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A MANUAL OF IMMUNITY FOR STUDENTS AND PRACTITIONERS

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A MANUAL OF
IMMUNITY

FOR STUDENTS AND PRACTITIONERS

BY

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DEDICATED TO

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OF LONDON,
WHOSE HOPEFUL, UNTIRING AND BRILLIANT
WORK HAS BEEN AN INSPIRATION TO THE
AUTHOR AND MANY OTHERS

PREFACE

OWING largely to the almost wanton introduction of new terms, the science of Immunity has acquired an aspect of complexity which may well suffice to scare the busy general practitioner from any attempt to study the subject. This is surely a misfortune. The day has gone by when the things of Immunity could be entirely relegated to the laboratory. During the last twenty years, this science has been the most living branch of Medicine, and in these days of Sero-diagnosis and Vaccine and Serum Therapy, it is obvious that the physician can no longer be a mildly interested on-looker.

The doctor who has to inject a vaccine or an antitoxin, or who has to communicate to his patient the significance of a positive Widal or a positive Wassermann reaction, must either equip himself with a knowledge of the fundamental facts of Immunity, or be forced into the position of a passive transmitter of ideas which are entirely beyond his grasp.

GLASGOW, 14*th October*, 1912.

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CHAPTER I.

HISTORICAL SURVEY.

By "Immunity" is understood the privilege which confers on an animal exemption from injury by a particular micro-organism or a particular toxin, which is pathogenic for other animals of its own or of a different species. The science which treats of the causes and the mechanism of such exemptions is known as the science of Immunity.

Immunity may be "natural" or "acquired."

Natural Immunity is that possessed normally by certain individuals or certain species. For example, one finds individuals who, in spite of repeated exposure to an infectious disease, such as Scarlet Fever, Tuberculosis, Small-pox, etc., never contract the infection; they are protected by some peculiarity of organisation which renders them invulnerable. Then certain species of animals are immune to infectious diseases which affect other species—man is immune to Rinderpest, most animal species are immune to Syphilis, a man can work with impunity with Nagana Trypanosomes which will kill a rat with certainty in three days, and so on.

Acquired Immunity is that which an animal attains as a result of having successfully passed

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through and overcome an infection. As is well known, one attack of Scarlet Fever, Measles, Whooping Cough, Small-pox, etc., confers a more or less enduring immunity against a second attack of the same disease. Acquired immunity may also be the result of various technical procedures, such as vaccination and serum therapy.

The simplest and the most interesting way in which to get a clear view of the present position of Immunity is to follow the main discoveries in their historical sequence and note how one led to another. With such an infant science the process need not be tedious.

The science of Immunity really begins with Pasteur and dates its origin from about the year 1879. This, of course, does not mean that before Pasteur we had no "Immunisators." Before Pasteur we had Jenner, and before Jenner we had the Gloucestershire dairy folk, who knew that Cow-pox protected them against Small-pox; and even before these we doubtless had the mother who wilfully exposed her child to measles in order to immunise it against a later attack.

Even in ancient history we can trace the germ of our modern ideas on immunisation. Pliny the Elder recommended the livers of mad dogs as a cure for rabies, thus coming amazingly near to the truth of Pasteur's great discovery.

It was a dogma of Hippocrates that the factor which produces a disease is also capable of curing it. We have the same idea in the mythological tale of how Telephos cured his wound by the application of rust from the sword which inflicted it, and in the story of how Mithridates, King of Pontus (B.C. 120), immunised himself against poisons by drinking the blood of ducks which had been treated with the corresponding toxic substances.

Processes of immunisation against snake venom were practised by many savage tribes in the earliest times, and preventive inoculations against Small-pox have been widely recognised in several parts of Asia for very many centuries.

As far as is known, however, all these early immunisators were the most absolute empiricists, and to Pasteur must be ascribed the honour of carrying out the first scientifically reasoned immunisation. In the *Life of Pasteur* (René Vallery-Radot), it is stated that, through all his researches in what might have been considered "abstract science," the discovery of some means of conferring protection against infective disease was the constant quest of this brilliant bacteriologist. Quite by accident—or so it seemed—he stumbled on the discovery that the virulence of micro-organisms is subject to attenuation, and with that knowledge the key was in his hand. He found that he was able to use these attenuated micro-organisms for inoculation, and that thereby he could produce immunity against the non-attenuated, *i.e.* virulent micro-organisms of certain infective diseases. The first experiments were with fowl cholera, and these were followed by analogous experiments which led to the discovery of the vaccines against anthrax, swine erysipelas and rabies—

discoveries which were soon to find such important practical application. The antirabic vaccine is probably more largely responsible than any other single factor for extending the knowledge of the possibility of scientific immunisation.

Let us now briefly consider the various theories of Immunity that have succeeded each other since the days of Pasteur.

1. PASTEUR'S own theory was that a micro-organism, by growing in the animal body, used up all the materials necessary for its maintenance, that these materials consequently became exhausted and that the micro-organism was eventually forced to retire for lack of proper nourishment—was, so to speak, starved out. This is known as the "Exhaustion Theory."

2. In opposition to this the "Retention Theory" was offered by *Chauveau*, who held that bacterial toxins, produced by the growth of bacteria in the organism and accumulating in the blood, have an inhibitory action on the invading bacteria.

Various other views were put forward to explain the known phenomena in natural and acquired Immunity, but all were framed on the most meagre fabric of facts.

3. It was not until *Metchnikoff's* famous address, given in Odessa in 1883, that the world got a glimpse of the true direction in which it must look for light on the dark problems of Immunity.

Metchnikoff was a zoologist, and to this fact we owe much. By his researches along the lines of Comparative Pathology, he was able to arrive at his theory of the rôle of Phagocytosis in the defence of the human body against micro-organisms. By studying the simplest unicellular organisms, he was able to convince himself that, when these are face to face with a hostile bacterium, they behave just as they do before a food particle. An amoeba, for example, in presence of a nutrient particle, throws out protoplasmic processes (pseudopodia), and by means of these surrounds the particle and conveys it into its own interior, where it gradually undergoes a process of intracellular digestion, through the action of a ferment, the residual undigested part being eventually extruded.

The same mechanism serves for protection against infective parasites—these being taken into the protoplasm of the cell and disposed of by intracellular digestion.

Metchnikoff then expounded the idea that this faculty of unicellular organisms has been hereditarily transmitted to the higher animals, where it resides in the amoeboid cells of mesodermic origin. In the vertebrates, nutrition is, of course, no longer carried on by means of this simple process, but takes place by a process of extracellular digestion in a specialised alimentary canal. According to Metchnikoff's theory, however, even in the vertebrates the "phagocytes" still retain the functions of their prototype, the amoeba. They are able to take up particulate matters and to digest them intracellularly. This, Metchnikoff has traced very carefully in the process of inflammation and absorption of inflammatory exudates. In the absorption of extravasated blood also, it is these phagocytes that play the chief part by taking up the red blood corpuscles and other formed elements and digesting them. Similarly Metchnikoff traced the analogy between the method of disappearance of micro-organisms when injected into an immune animal and the disappearance of cells in inflammatory exudations or in extravasations of blood—in all cases the process is one of seizure by the amoeboid cells, followed by digestion in the cell interior. It is known that leucocytes contain several soluble ferments which may be set free when the blood is shed; *e.g.* "plasmase" or fibrin ferment. Metchnikoff holds that the phagocytes contain a ferment for the intracellular digestion of micro-organisms; this ferment he names "cytase."

This theory of Metchnikoff is treated more fully in the chapter on Phagocytosis.

It must be noted that Metchnikoff was the first to propound the notion of an active bodily defence against invading micro-organisms.

The publication of this theory brought down a storm of opposition, and for a time Metchnikoff's views seemed very unlikely to gain support.

4. In 1886, *Fodor* discovered that the blood of the rabbit is able to kill Anthrax bacilli in test-tube experiment. This "bactericidal" property he supposed to be entirely due to the fluid part of the blood—the cells were utterly ignored—and he proceeded on this uncertain foundation to build a "humoral" theory of Immunity. Whenever an animal showed immunity to any micro-organism, the

supposition was that it possessed, in its blood plasma, sufficient bactericidal substance to kill off the particular invaders. It was admitted that the phagocytes might ingest bacteria, but only provided the bacteria were dead—the rôle of the phagocyte was, at the most, that of scavenger.

This purely humoral theory of Immunity found many eager adherents.

5. *Hans Buchner* adopted and elaborated the theory. He believed the bactericidal action of the blood to be due to a special albuminoid substance which he called "alexin."

There were many difficulties in the way of accepting this purely humoral explanation of Immunity, based on the bactericidal power of the blood. For example, many instances were known where the blood possessed a high bactericidal power, and yet the animal was not thereby protected from infection by the micro-organism, and conversely, many cases of Immunity could be cited in animals whose blood showed no bactericidal action against the micro-organisms in question.

6. In 1890, *Von Behring and Kitasato* had made their famous discovery of antitoxins in the blood of patients suffering from Diphtheria and Tetanus. Von Behring then tried to base a general theory of Immunity on this discovery. He endeavoured to show that this antitoxic property is a *sine qua non* of acquired Immunity. Evidence very soon accumulated, however, to demonstrate that this antitoxic power is a factor only in exceptional cases of Immunity—chiefly in Diphtheria and Tetanus—and

that in the great majority of instances of Immunity, either natural or acquired, there is no antitoxic action of the body fluids.

7. Then, in 1894, *R. Pfeiffer* announced a discovery which seemed to offer substantial support to the "humoral" as against the "phagocytic" explanation of Immunity. He showed that when Cholera vibrios are introduced into the peritoneal cavity of a guinea-pig, which has been previously immunised against Cholera, these vibrios are transformed into granules and ultimately undergo complete solution in the peritoneal fluid (bacteriolysis). The phagocytes here seemed to have no share in the protection of the animal, and for a time the "humoral" school appeared triumphant. Metchnikoff, however, undauntedly set to work to uphold his views, and it was not long before he succeeded in showing that the so-called "Pfeiffer phenomenon" is, like the antitoxic property, limited to a few groups of bacteria (especially Vibrio Cholerae and Bac. Typhosus), and that, even with these, it only manifests itself after injury of the phagocytes. This damage to the phagocytes he named "phagolysis," and he showed that it took place after the injection of bacteria into the peritoneum, and also after the injection of many other substances, such as broth or even normal saline solution. If, by any artifice, phagolysis were suppressed (and Metchnikoff showed a method of accomplishing this), no granular transformation occurred; the vibrios were phagocyted in an unaltered condition. Metchnikoff further showed that the "Pfeiffer phenomenon" takes place

"in vitro" if an emulsion of Cholera vibrios is mixed with some immune cholera serum, and a small quantity of fresh peritoneal lymph is added. A hanging-drop preparation of the mixture shows the granular transformation, etc.

8. *Bordet* then made the important observation that when *perfectly fresh* immune cholera serum is added to an emulsion of Cholera vibrios "in vitro," the serum alone suffices to produce the Pfeiffer phenomenon, without requiring the addition of peritoneal lymph. When, however, the immune serum had stood in the laboratory for some time—as was the case with the serum used in the experiment of Metchnikoff just described—or when it had been heated to 55° C., it lost this power, and only regained it on the addition of a small quantity of fresh serum, normal or immune.

Bordet thus demonstrated that two substances are necessary to produce bacteriolysis; these he called "sensitising substance" and "alexine." The sensitising substance is specific, existing only in the immune serum and acting only on the homologous bacteria (bacteria against which the animal supplying the serum has been immunised). It resists heating for half an hour at 56° C. The alexine, on the other hand, is non-specific, occurs in practically the same amount in fresh normal serum and in fresh serum from vaccinated animals, and is rendered inert by heating to 56° C.

Bordet also showed that, by a common mechanism, the organism immunises itself, not only against bacteria, but against a great range of widely differing substances. For example, when he injected blood corpuscles of one animal into another of a different species, he obtained an immunisation reaction quite analogous to that observed after the injection of bacteria. He inoculated guinea-pigs with rabbit's blood, and showed that the guinea-pig's serum acquired the power of agglutinating the rabbit's red corpuscles and also of dissolving (lysing) them. These, as Bordet noted, are precisely the phenomena which occur when B. Typhosus or Vibrio Cholerae is injected into a guinea-pig—the guinea-pig's serum acquiring an agglutinating and a lytic power. Then Bordet went on to show that for this process of Haemolysis, as for that of Bacteriolysis, two substances are necessary—the " sensitising substance " and the " alexine."

9. With much ingenuity, *Ehrlich* endeavoured to explain the action of these two substances involved in bacteriolysis, by means of his " Side Chain Theory."[1] The sensitising substance he named " amboceptor " and the alexine he named " complement."

It seems a pity that Bordet's original terms should have been supplanted by names which belong only to theory, which beg the question and which are possibly altogether misleading; but they have now become part of the Immunity vocabulary in England and America, as well as in Germany.

10. *Metchnikoff* brought his phagocytosis theory into line with these new discoveries. The " complement " or " alexine " he regarded as a soluble

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[1] See Appendix, p. 171, which the student is recommended to read at this point.

ferment, a product of the phagocytes, liberated on destruction of these cells, but usually acting only within them (intracellular digestion). The " sensitising substance " or " amboceptor " he regarded as also a product of the phagocytes, which, however, does not act within these cells, but is given off into the fluid surrounding them.

11. The next phase in the development of the Immunity controversy was inaugurated by the discovery of the protective power of serum as a factor distinct from bacteriolytic, bactericidal, agglutinating or antitoxic properties. Many observers had shown that human blood serum can protect a guinea-pig against a lethal dose of the Cholera vibrio, provided the serum is injected twenty-four hours before the bacteria. The same was found in the case of B. Typhosus and many other bacteria. This protective action, though much more marked with immune serum, is found also with normal serum.

Metchnikoff and *Besredka* explain this protective effect as due to a stimulation of the phagocytes by the serum. The substances, which cause the stimulation, they called " stimulins." As a protective serum may come from an animal which is not itself refractory to the disease, Metchnikoff argues that it is to the reaction of the cells in the animal receiving the serum that its salvation is due.

12. Until about the year 1895 these two theories of Immunity, the " humoral " and the " cellular," were maintained by the opposing schools with a zeal which savoured almost of bigotry. In this year, however, *Denys* and *Leclef* called attention to the

fact that the phagocytosis of bacteria "in vitro" is much facilitated if one adds a small quantity of the serum of an animal which has been immunised against the particular micro-organism in question. From this time, a new school arose, which has tended to reconcile the opposing views by showing that each contained part of the truth. (See section on Opsonins.)

Denys and Leclef, in the course of some researches on immunity against the streptococcus, inoculated streptococci subcutaneously into the ear of normal and immunised rabbits, and examined the fluid of the resulting oedema by frequent puncture and removal of fluid. They found that, in the normal animal, there is a rapid increase of the bacteria and evidently no phagocytosis; in the immune animal, on the other hand, phagocytosis goes on so rapidly that the streptococci do not get the opportunity to multiply, and are soon destroyed and entirely disappear.

In subsequent experiments, Denys and Leclef endeavoured to apportion the share taken in this immunisation process by the various factors concerned. They compared leucocytes from a normal animal with leucocytes from an immune animal, and found that both behave practically alike—at least in experiments "in vitro." In presence of anti-streptococcic serum, both normal and immune leucocytes show the most active phagocytosis; in presence of normal serum, each kind of leucocyte shows only the feeblest phagocytic activity. They therefore conclude that no difference, in respect of phagocytic power, exists between the leucocytes of a normal and those of an immunised animal. It is to the presence of the specific serum that the difference is due.

13. In the year 1903, *Wright* and *Douglas* announced the discovery of a new substance in the blood serum, which, according to them, is the essential element in the process of phagocytosis; this substance they named "Opsonin." Without opsonin, they hold, there is no phagocytosis.

The opsonin attaches itself to the bacteria and thereby modifies them, so that they become much more phagocytable—more savoury for the phagocytes. It is present in normal serum, and is increased in amount during the process of immunisation.[1]

14. *Neufeld* and *Rimpau* (1904) pointed out that in immune serum there is a substance which causes the bacteria to be phagocyted, but which differs from Wright's opsonin in being thermostable—resisting temperatures of 59° C., while the opsonin is destroyed at 56°. This thermostable substance they named "Bacteriotropin."

15. *Bail* lays great stress on the importance of the characteristics of the micro-organism as a factor in Immunity. According to him, before a bacterium can multiply in an animal and manifest pathogenic power, it must be able to paralyse the protective mechanism of the animal; this it does by the production of certain attacking substances, the so-called "Aggressins."

It becomes daily more obvious that, up to the present, too little attention has been paid to the importance of the micro-organism and its variability in all immunity reactions. The bacteria are able to immunise themselves against the deleterious influences of their host ; virulent strains and " resistant races " are evolved, and these complicate enormously the practical problems of immunisation. (This aspect of the question is discussed more fully in Chap. IV.)

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[1] This question is fully discussed in Chap. II. under "Opsonins."

CHAPTER II.

THE BODY FLUIDS AS A FACTOR IN IMMUNITY.

In the preceding chapter something has been said of the part played by the various factors in Immunity—the body fluids, the body cells and the invading bacteria. These factors will now be considered individually, as this seems the most logical method of dealing with the subject.

In the present chapter the various known antibodies, which are found in the blood serum, will be taken up consecutively and treated as shortly as is consistent with the setting forth of the important practical points.

For definitions of terms which may be obscure, the reader is referred to the glossary at the end of the book; a thorough grasp of the meaning of these technical terms is a necessary preliminary to the understanding of any chapter on the humoral substances in Immunity.

1. ANTITOXINS.

These are antibodies produced in an animal in response to the injection of bacterial toxins. A *bacterial toxin*, strictly speaking, is a secretion product of the bacteria, which is given off into the medium in which they are growing. It is characterised by the fact that it requires a certain incubation period before manifesting its effects when introduced into the animal body, and also by the fact that it can act as an antigen—giving rise to the formation of an antibody, the "Antitoxin." This antitoxin combines with the toxin[1] and, according to Ehrlich, neutralises it in conformity with the law of multiple proportions—*i.e.* if one dose of toxin is neutralised by a certain quantity of antitoxin, double that dose will be neutralised by double the quantity of antitoxin. In all these respects the true bacterial toxins differ from ordinary chemical poisons.

Only certain bacteria, as far as is known, produce such toxins (" true toxins ") ; these bacteria are B. Diphtheriae, B. Tetani, B. Dysenteriae (Kruse-Shiga), B. Botulinus, and, to a less extent, the Meningococcus, Vib. Cholerae and B. Typhosus.[2] The clinical picture, in the case of an infection with one of these toxin-producing germs, differs widely from that of a septicaemia, where the bacteria themselves invade the blood. Even in the most severe and fatal cases of Tetanus, one never finds the bacillus in the blood.

In Diphtheria, the bacteria are largely confined to the region of the throat ; the throat lesions may be severe or may be slight, but in any case they are obviously insufficient to account for the alarming constitutional symptoms and sequelae. These, as has been proved, are due to the toxins secreted by the bacteria as they grow in the throat and are thence absorbed into the circulation.

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[1] Bordet, Craw, Landsteiner and others contest this view.

[2] It is still, however, a disputed point whether the toxins of Vib. Cholerae and B. Typhosus are " true toxins " or endotoxins.

In Dysentery, the intestine is the site where the bacteria form their toxins and from whence these reach the blood stream.[1]

To produce the toxin in the laboratory, bouillon cultures of the bacteria are made and are allowed to grow for a certain number of days. The cultures are then filtered and the toxin is obtained in the filtrate.

In 1890, V. Behring and Kitasato published their discovery of the Diphtheria and Tetanus antitoxins. They showed that the blood of a rabbit, which has been immunised against Tetanus, is able to destroy the effects of the Tetanus toxin. This property persists even after the blood has been transfused into other animals.

The substance in the blood, which is able to destroy the effect of the toxin, is called "antitoxin."

The process of obtaining antitoxin is briefly as follows :

An animal must be actively immunised against the particular toxin in question, first by the injection of a quantity of toxin smaller than the minimum lethal dose, then by larger doses as it becomes more and more tolerant. Eventually the animal is able to endure, without injury, the effects of injections many times greater than the lethal dose. Its blood then contains considerable quantities of antitoxin.

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[1] This holds only for the Shiga-Kruse type; the Flexner type of bacillus does not form any true toxin.

To show that the blood contains a substance which is capable of neutralising the effect of the toxin, it is only necessary to mix a quantity of the serum with a lethal dose of toxin "in vitro" and then inject the mixture into a guinea-pig. The animal remains unhurt—protected by something in the borrowed serum; this something is called "Antitoxin." The serum containing antitoxin is able, when injected into an animal, to protect it against an otherwise certainly fatal dose of toxin subsequently injected; it is also able to *cure* an animal already suffering from the effects of a toxin. To this latter fact is due its extraordinary value in serum therapeutics.[1]

Antitoxins are also formed in the animal body against many toxins of vegetable origin—ricin, abrin, robin, etc.—and against some toxins of animal origin, *e.g.* snake venom.

The action of an antitoxin is specific; Diphtheria antitoxin acts only against Diphtheria toxin, Tetanus antitoxin only against Tetanus toxin, and so on.[2]

2. AGGLUTININS.

The *Agglutinins* are substances which possess the power of causing bacteria, suspended in a fluid, to collect together into clumps, which gradually separate out from the fluid and collect at the bottom.

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[1] For the methods of producing special antitoxic sera, see page 78.

[2] At this point the student is advised to get a grasp of the Ehrlich Side Chain Theory (Appendix), which offers a hypothetical explanation of the constitution of toxins and antitoxins and of their mode of action and interaction.

Bordet (1895) was the first to call attention to the phenomenon known as "Agglutination." He showed that, when a small quantity of the serum of an animal, immunised against Cholera, is added to an emulsion of Cholera vibrios in salt solution, the vibrios very quickly lose their motility and collect into small clumps.

To Gruber and Durham, however, belongs the credit of recognising the significance of agglutination as an Immunity reaction, and of proving the specificity of the substances which cause it.

They showed that the serum of animals, which had been immunised against B. Typhosus, possessed the power of agglutinating typhoid bacilli in a uniform suspension—that the bacilli became massed together into little granules and eventually sank to the bottom, leaving the supernatant fluid quite clear.

If an emulsion of Cholera vibrios were used with the serum of an animal immunised against Cholera, the same phenomenon was observed.

Cultures made from the precipitate showed that the bacteria were still alive after agglutination.

This phenomenon may be studied by mixing the bacterial suspensions with varying dilutions of serum in test tubes and observing the effect with the naked eye. It may also be observed in " hanging drop " preparations, under the microscope—a method which is especially useful when only a small quantity of serum is available. Microscopically it will be seen that motile bacteria soon lose their motility, then become matted together and finally form into clumps.[1]

These Agglutinins in the immune serum are always specific, that is to say they act only on the bacteria against which the animal furnishing the serum has been immunised.

If normal serum, instead of immune serum, is added to the bacterial emulsion, a certain amount of agglutination will still be observed; it is, however, very incomplete—the reaction being much less intense than in the experiment where immune serum is used. If, now, a series of graduated dilutions of the normal and of the immune serum are made, and the agglutinating effect of each dilution is tested, a very important distinction at once appears: when diluted very slightly, the normal serum loses its agglutinating power, whereas the immune serum retains this power even when diluted to a high degree.

Widal was the first to turn these facts to practical account in the diagnosis of disease, and thereby to gain the distinction of introducing sero-diagnostic methods into medicine. He found that the serum of patients suffering from Typhoid Fever acquires a high agglutinating power for B. Typhosus, and that, as this phenomenon generally manifests itself comparatively early in the disease, its recognition may be of great diagnostic importance.

In the "Widal reaction," advantage is taken of a *quantitative* difference in the agglutinin content of immune as compared with normal serum. For example, in a given experiment a Typhoid immune serum may agglutinate B. Typhosus in a dilution of 1 : 100 and may also agglutinate other bacteria, such as Vib. Cholerae, up to a dilution of 1 : 10 ; on the other hand, a normal serum or an anticholera serum may not agglutinate B. Typhosus in a greater dilution than 1 : 10. In technical terms this is expressed by saying that the agglutinating power of an immune serum against the homologous bacteria is very much higher than the agglutinating power against heterologous bacteria. (" Homologous bacteria " = bacteria used in immunising the animal to obtain the serum ; " heterologous bacteria " = other than those of the species used for the immunisation.)

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[1] For the technique of agglutination experiments, see pages 114-117.

It is clear then :

(1) that an immune serum may be used in a much higher dilution than a normal serum against the homologous bacteria, and

(2) that, in order to produce agglutination, the immune serum may be used in much higher dilution for the homologous bacteria than for the heterologous. As will presently be shown, these two facts are made use of in a practical way :

A. in the diagnosis of various bacterial diseases, and

B. in the differentiation of certain bacteria.

PARTIAL AGGLUTININS.

One more point is to be observed about this agglutinating power (" agglutination titer ") of an immune serum : whilst the immunisation has so greatly increased the agglutinating power for the corresponding bacteria, it has, at the same time, increased (though to a much less degree) the agglutinating power towards other closely allied bacteria. For example, a Typhoid immune serum possesses, besides its greatly increased agglutinating power for B. Typh., an agglutinating power for B. Paratyph., which, although not nearly so much augmented, is nevertheless notably above that of the normal serum. This is explained by the very close biological relationship of these bacteria, together with the fact that the agglutinin-producing substance (agglutinogen), in any given bacterium, is a complex and not a single chemical substance. If, for example, the Typhoid agglutinogen is composed of various elements, A, B, C, D, it is quite conceivable that the protoplasm of the nearly related Paratyphoid bacillus might contain some of the same elements—might, let us say, have agglutinogens B, C, D, E. Now the serum of an animal immunised against Bac. Typh. would contain four separate agglutinins for these four agglutinogens, and the agglutinating power of the serum would be represented by the sum of these four. But, as the Paratyph. bacillus possesses three of the same agglutinogens, the agglutinating power of the serum on this bacillus, though not so great as on the Typh. bacillus, will still be considerable.

HAEMAGGLUTININS.

These are substances which agglutinate red blood corpuscles. They are similar to the bacterial agglutinins and are produced (along with haemolysins) in the serum of an animal which is inoculated with red blood corpuscles from another species. When a serum containing haemagglutinins is mixed with a suspension of the corresponding red blood corpuscles, it causes these to collect into clumps and sink to the bottom, just as a typhoid immune serum does with typhoid bacilli.

Inagglutinable Races of Bacteria.

Certain races of bacteria, especially when newly cultivated from the animal body, may show themselves insusceptible to the action of the agglutinins. As a rule, however, a few generations of sub-culture, on artificial media, will serve to restore their agglutinability.

It should be noted that agglutinins act on dead as well as on living bacteria. Neisser introduced the use of emulsions killed by formalin for the microscopic agglutination tests.

Bacteria killed by heat or by chloroform are similarly agglutinable.

The method of performing the Widal test by means of the "Ficker Diagnosticum," with dead bacteria, is useful for the general practitioner (see pages 115-116).

Rôle of Agglutinins in Immunity.

This rôle is not quite easy to determine. The serum of animals, which have acquired Immunity, as a rule shows a certain amount of agglutinating power for the corresponding bacteria. In certain cases, however, this power is entirely lacking and yet the animal enjoys Immunity. For example, the serum of the dog, which is immune to anthrax, is much less agglutinative for the Bac. Anthracis than the serum of the ox, which is susceptible to the disease.

It is also to be noted that the agglutination of bacteria does not prevent their growth and evidently does not lessen their virulence.

Many observations go to show that the agglutinating power of the serum of an animal gives no indication of the degree of the animal's immunity against the particular micro-organism. For example, relapses may occur in Typhoid Fever at a time when the agglutinating power of the patient's blood is at its highest. Numerous similar examples might be cited. It is only necessary, however, to state that agglutinins are now definitely regarded as playing only a very insignificant part in Immunity. Their diagnostic value is as an "accompaniment"—from which one may deduce the presence of the more important factors.

THE MECHANISM OF AGGLUTINATION.

Gruber's idea of the mechanism of this phenomenon was that the agglutinin changed the bacterial membrane so as to render it more viscous, and that this increased viscosity caused the bacteria to adhere and form clumps.

Bordet's view is that agglutination is a phenomenon of molecular physics—that the serum acts on the bacteria and permits their agglutination by altering the relations of molecular attraction between them and the surrounding fluid. According to him the phenomenon of agglutination should be divided into two phases. The first is the period when the isolated elements (bacteria, blood corpuscles, etc.) are affected by the agglutinin, and the second a period of agglutination, properly so-called. In the first phase, the agglutinin acts on the bacteria or cells for which it is specific, and, according to the hypothesis, alters the relations of molecular attraction between them and the surrounding fluid. The second phase, the clumping, etc., is then brought about by the presence of salt. In experiments, arranged so that salt is excluded, no agglutination takes place, although the bacteria have already been acted on by the suitable agglutinin. This second phase, therefore, would be a purely physical phenomenon. Bordet instances the precipitation of clay as an analogous case: if a little salt is added to a fine emulsion of potter's clay in distilled water, the clay immediately clumps and falls to the bottom. The resemblance between these flakes and the clumps of agglutinated bacteria is very striking.

Joos opposes this view of Bordet; he denies that the salt plays a purely physical part in the agglutination, and considers that it enters into chemical combination.

ORIGIN OF AGGLUTININS.

Researches have been carried out for the purpose of determining the site of manufacture of agglutinins, but, so far, the results are inconclusive. Some authors believe the spleen to be the chief source, others ascribe this function to the blood vessels.

Agglutinins do not appear in the serum immediately after inoculation, but require an incubation period of two to four days.

The normal foetus possesses no agglutinins; the agglutinins of normal serum must be formed after birth.

PRACTICAL APPLICATIONS.

The agglutination reaction is used for two purposes:

A. For the diagnosis of disease, and
B. For the differentiation of bacteria.[1]

It now remains to take up these practical uses in some detail, as each presents certain peculiarities.

A. THE AGGLUTINATION REACTION IN THE DIAGNOSIS OF INFECTIVE DISEASES.

1. *Typhoid Fever and Paratyphoid Infections.* In Typhoid Fever the Widal reaction may be positive as early as the third day; usually, however, the positive reaction is only obtained somewhat later—from the fifth or seventh day—and occasionally it appears first during the stage of convalescence.

The reaction is sometimes quite absent where the diagnosis is confirmed by cultivating typhoid bacilli from the blood; it would thus appear that certain individuals are incapable of producing agglutinins.

The reaction usually remains positive for several weeks and then gradually returns to normal. Occasionally cases are seen in which the reaction remains positive for months or even years after the attack of Typhoid Fever.

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[1] The technique is given on pages 114-117.

The blood of "Typhoid carriers" also sometimes gives a positive reaction, although the "carrier" appears quite well.

An infection with Bac. Paratyphosus "A" or "B" may be difficult to distinguish from Typhoid Fever owing to similarity of symptoms and the confusion of the Widal test by the presence of "partial agglutinins" in the serum. As has been already explained, Paratyphoid sera generally agglutinate B. Typh. only slightly, but Typhoid sera may have a very high agglutinating power for Bac. Paratyph. In doubtful cases the Castellani experiment (see Citron: "Immundiagnostik und Immuntherapie," p. 116) should be used.

The Widal reaction may only be regarded as positive if the Bac. Typh. is agglutinated by the patient's serum in a dilution of at least 1 : 100. Occasionally the sera of healthy people may agglutinate B. Typh. in dilutions of 1 : 50.

It must also be remembered that a positive reaction is sometimes obtained in other diseases—in Miliary Tuberculosis, Malaria, Malignant Endocarditis, Carcinoma.

As a source of fallacy, it is to be borne in mind that a mixed infection (*e.g.* with Staphylococcus Albus, Pneumococcus or Streptococcus) in Typhoid Fever may yield a negative Widal reaction.

2. *Cholera.* The agglutination test has so far proved of very doubtful aid in the testing of a Cholera serum.

Here the Pfeiffer experiment, or the identification of cholera vibrios in the stools, by the aid of an immune serum, is of much greater value for clinical diagnosis.

3. *Cerebro-Spinal Meningitis.* The agglutination test has here an admitted value. The reaction is regarded as positive if agglutination occurs within a few hours with a 1 : 10 dilution of the serum. (It is seldom that a Meningococcic serum agglutinates in a dilution higher than 1 : 50.)

Difficulty may sometimes occur through the inagglutinability of certain races of the Meningococci.

4. *Tuberculosis.* The agglutination test has so far achieved no recognised value in the diagnosis of Tuberculosis. The technique is difficult and the reports of various observers are contradictory.

Many strains of tubercle bacillus are almost, or altogether, inagglutinable. Then the preparation of a homogeneous emulsion is no easy matter. Koch recommended the agglutination test for the estimation of the degree of immunity conferred by Tuberculin treatment. He found that the serum of patients treated with Tuberculin acquires a greatly increased agglutinating power. However, the agglutinating power of a serum, as is now well recognised, cannot be regarded as indicating in any way the degree of immunity, and so the value of this experiment disappears.

5. *Plague.* The agglutination reaction is here very marked, but its value in the diagnosis of Plague is minimal as the augmented agglutinating power does not appear till relatively late in the disease—about the ninth day. It may occasionally be useful for

deciding whether a patient in the convalescent stage has really suffered from Plague.

B. THE AGGLUTINATION REACTION FOR THE DIAGNOSIS OF BACTERIA.

1. *The Typhoid and Paratyphoid Groups of Bacilli.* Here one employs a known serum to identify an unknown micro-organism. (Such a serum is readily obtained by the intravenous injection of dead cultures into an animal.)

It is best to use the macroscopic method (page 114).

The agglutinating serum to be used in the test must be previously titrated against an easily agglutinable, known strain of the micro-organism.

The " partial agglutinins " constitute the difficulty in differentiating the very numerous members of these groups from each other, but if a highly agglutinating serum be used and the test carried to the point of determining the highest dilutions which agglutinate the bacteria, it will, in most cases, be possible to separate the various allied micro-organisms by this test.

Another difficulty is offered by the occurrence of inagglutinable or very slightly agglutinable strains. Typhoid bacilli, when newly cultivated from the organism, often belong to this class; the Bac. Typhosus isolated from post-typhoid abscesses has also been found to resist agglutination. The agglutinability is usually restored by subculturing on artificial media. By means of this test, it is possible to differentiate B. Typhosus, B. Paratyphosus "A" and " B," B. Enteritides Gärtner, etc.

2. *B. Dysenteriae.* This may be separated from the pseudo-dysentery bacilli by means of the agglutination test with immune serum.

3. *B. Tuberculosis.* A serum which is highly agglutinating for the tubercle bacillus may be obtained by the artificial immunisation of animals.

The question as to whether the different races of Bac. Tuberculosis can be separated by means of this test, and whether the bacillus can be differentiated from other acid-fast bacilli, remains still to be decided.

4. *Pneumococcus.* This bacterium may be agglutinated by fairly high dilutions of immune serum; the reaction, however, possesses so far no clinical significance.

5. *Meningococcus.* The Diplococcus of Weichselbaum can be identified by means of a specific agglutinating serum. Normal serum shows no agglutinating effect on the Meningococcus.

It is to be noted that a specific meningococcic serum contains also partial agglutinins for the gonococcus, and a specific gonococcic serum contains partial agglutinins for the Meningococcus.

6. *B. Pestis.* In the diagnosis of Plague, the agglutination reaction is very valuable.

An antiplague serum agglutinates the bacilli; the agglutination is absolutely specific, and its significance is not confused by any question of "partial agglutinins."

3. PRECIPITINS.

Perhaps no chapter speaks more significantly for the great practical value, and the still unfathomed possibilities of this infant science of Immunity, than does the chapter on the precipitins.

Here there is a contribution for the Medical Jurist, the Anthropologist and the Food Inspector, as well as for the Bacteriologist, the Physician and the Patient.

A discovery which can elucidate so many important questions—from the kinship between the apes and man to the particular adulteration of a sausage—is bound to compel attention. A glance at the awe-inspiring list of the literature on this subject will show how very real has become the attention.

DEFINITION.

The *precipitins* are specific substances, found in the serum of animals immunised against bacteria or against solutions of animal or vegetable albumins, which possess the power of producing a precipitate in a clear solution of the particular albumin or in a clear culture filtrate of the bacteria against which the animal has been immunised.

The specific substances in the immune serum are called "Precipitins," the albumin used as antigen is called "Precipitinogen."

HISTORY.

Kraus (1897) was the first to note the phenomenon. He observed that by taking the serum of animals which have been immunised against Cholera, and adding it to a clear filtrate obtained from a culture of the Vibrio Cholerae, he obtained a precipitate in this fluid.

This reaction is specific, *i.e.* occurs only with the homologous bacteria. For example, the Cholera-immune serum does not produce a precipitate in a filtered culture of Bac. Typhosus, and similarly a Typhoid-immune serum does not produce a precipitate in a filtered culture of Vib. Cholerae.

Then *Tchistovitch* found that the serum of rabbits, which had been inoculated on several occasions with eel serum, caused a cloudiness to develop when mixed with a small quantity of this serum.

This proved to be a phenomenon of a general kind : when an animal A is injected with the serum of an animal B, the serum of A acquires the power of producing a precipitate when added to the serum of B.

Almost at the same time *Bordet* produced a so-called lacto-serum (*i.e.* anti-milk immune serum). He gave to rabbits intraperitoneal injections of milk, which had been partially sterilised by heating to 65° C. After three or four injections at proper intervals, the rabbits yielded a serum which showed the power of producing a precipitate in milk. When this serum is mixed with milk small particles appear, and these gradually form larger flakes and sink to the bottom of the fluid. If this is passed through filter paper, the filtrate is quite clear and limpid and has lost the white cloudiness of normal milk.

Wladimiroff was the first to use the bacterial precipitin reaction for diagnostic purposes. He showed that the serum of a horse suffering from Glanders could produce a precipitate when added to a clear filtrate of a culture of Bac. Mallei.

Uhlenhuth found that, by using sera of animals immunised against various egg albumins, it was possible to discover the kind of egg (hen, goose, pigeon, etc.) from which the albumin had come.

This was a distinct advance on any chemical procedure, as no known test was able to distinguish between these different kinds of albumin.

The reaction is extraordinarily delicate; even in dilutions of 1 : 100,000 it is possible to detect the specific albumin. The ordinary chemical tests for albumin are crude in comparison.

Soon after, it was found that the precipitin test could offer itself as a valuable implement to the Medical Jurist.

Uhlenhuth and Wassermann found that by injecting rabbits with the blood of man or animals, sera could be obtained which gave a precipitate in the serum of the blood used for the injection, but not in that of other kinds of blood.

This test applied not only to specimens of fresh blood, but to old, dried-up blood spots.

This method has already proved of great value in numerous legal cases, and it is officially employed in the Courts of Justice in Germany and Austria. An example will illustrate its possibilities: consider the case where the charge of murder against a man may be based on the discovery of certain blood-stained articles in his possession. Now, even if these spots are old and dried, or partially decomposed, and whether they are on cloth, wood, steel, or other substance, the precipitin test can discover from what kind of animal the blood has come. If, in the given case, it is proved to be ox blood, then the charge against the man must fall to the ground.

Much interest from the scientific point of view attaches to the fact that the close relationship between certain species of animals expresses itself in this precipitin reaction. For example, the serum of an animal, immunised against human blood, gives also a precipitate in the serum of apes.

Embryology, palaeontology and comparative anatomy may have taught us the same thing, but it is rather a shock to realise that man's kinship to the monkey goes so far as a " blood relationship " !

Similar relationships can be shown between the blood of the horse and the ass, the sheep and the cow, the hare and the rabbit, the hen and the pigeon, etc.

These results are analogous to those described in the section on "Agglutination," under the heading of " Partial Agglutinins."

In that case we had to deal with the partial agglutination of closely allied bacteria by specific agglutinins ; here we have to deal with the partial precipitation of closely allied albumins by specific precipitins, and the explanation is probably the same (see page 22).

An immune serum, produced by the injection of the serum of a certain animal, gives a precipitate also with the juices of the various organs of that animal. The only exception to this rule is the albumin of the crystal lens of the eye, which gives no precipitate with the anti-blood immune serum. Further researches show that the same albumin exists in the crystal lenses of all kinds of animals from fishes to man (Romer has founded on this point some interesting work on the etiology and treatment of cataract).

By carefully freeing the organs from all their contained blood and then using organ extracts for inoculation, it is possible to produce sera which give a precipitate only with the particular kind of organ extract (liver, kidney, etc.), and not with extracts of the other organs of the same animal.

Maragliano applied this to the diagnosis of malignant tumours. By inoculating rabbits with cancer juice, he claims to have produced a serum which always gives a precipitate with the albumin of a cancerous tumour.

The precipitin test has also been used for the detection of minute traces of albumin in the urine.

The test has likewise proved of very great value in Food Inspection, as it permits the detection of a large variety of substances used for adulteration. The serum of a rabbit, immunised against horse flesh, gives a precipitate in a watery extract of horse flesh, but not in similar extracts of the flesh of other animals. Similarly an anti-dog precipitating serum gives a precipitate only in extracts of dog flesh, and so on.

By means of these specific sera, the presence of horse, dog, or cat flesh in mince meat or sausages can be conclusively demonstrated.

The age of the material under investigation—blood, meat, etc.—does not influence the reaction. Uhlenhuth was able to employ the test with accuracy on organs forty years old. Tests with ancient Egyptian mummies, however, gave a negative result.

A further practical use has been made of the precipitin test for the purpose of distinguishing different kinds of milk and the origin of different kinds of cheese.

Various other substances, such as butter, margarine, lard, etc., can also be classified and diagnosed by this method, provided they contain a certain amount of soluble albumin.

Most authorities are agreed that the precipitins are identical with the agglutinins.

SITE OF PRODUCTION OF PRECIPITINS. This is still undecided. Kraus and Levaditi tried to show that the leucocytes are the main source. It has more recently been held that both agglutinins and precipitins may be produced by the endothelial cells of the blood vessels.

The precipitin test for the diagnosis of bacteria is specific and just as reliable as the agglutination test. It is absolutely necessary, however, to titrate the serum, and to work carefully with high dilutions, as an immune serum can give a precipitate in a culture filtrate of a nearly related bacterium, as well as in that of the homologous germ. It will always be found, however, that the homologous culture gives a precipitate with a serum diluted beyond the point at which all precipitation with the heterologous filtrate absolutely fails.

Von Eisler was able to differentiate Bac. Typh. from the various closely allied bacteria by this test.

Kraus was able to separate the Koch Cholera vibrio from other related vibrios by using an immune Cholera serum.

For clinical diagnosis, however, the precipitin reaction has very little practical value, as the agglutination generally covers the same ground, and is much simpler in technique.

Metchnikoff regards the production of immune precipitins as a digestive reaction.

4. BACTERIOLYSINS.

The Bacteriolysins are substances which "lyse" bacteria, that is to say they cause them to alter their shape, and become shrivelled up and eventually converted into granules, at the same time robbing them of their motility and certain of their staining properties. The typical experiment to demonstrate this is the *Pfeiffer* experiment, and the transformation is known as the Pfeiffer phenomenon.

It should be noted that all amboceptors do not show a lytic action; this seems to depend rather on the constitution of the bacterium than on the amboceptor. For example, an antimeningococcic serum may contain an abundance of amboceptors (demonstrable by complement fixation), but the serum causes no bacteriolysis of the meningococcus, which is probably protected by a very resistant enveloping membrane.

It has already been noted that until the year 1894 no definite idea had been formed of the part played by the fluids of the body in the defence against aggressive bacteria. Metchnikoff had maintained his phagocytic defence as all-sufficient, and then, later, V. Behring had endeavoured to establish the antitoxins in the seat of honour as the most important element in Immunity. The limitations of these views, however, were soon made evident.

The bactericidal properties of the blood had been known for some time. Fodor, for example, had shown that rabbit's serum can kill Bac. Anthracis in test-tube experiment. These bactericidins were then hailed as giving the true key to the situation: when pathogenic bacteria enter the body, if they encounter sufficient of these bactericidal substances to destroy them all, the animal is immune and remains uninjured; if they do not encounter sufficient bactericidins, they multiply and produce their dire results. This explanation also had the recommendation of a most delightful simplicity—the sort of simplicity that appeals to some practitioners of the present day, who still fondly imagine that the one way to deal with all bacteria is by means of a carbolic or boracic solution! Unfortunately, however, the problem of Immunity is not quite so simple. An animal may die of an infection, although possessing in its blood a considerable quantity of bactericidal substances against the particular bacterium, and, on the other hand, an animal may be immune to a micro-organism although its blood contains no substances which are bactericidal for that micro-organism.

The fact that the white rat, which is naturally immune to Anthrax, possessed, in its blood, substances which could destroy Anthrax bacilli with great rapidity, was adduced in support of this view of the importance of the Bactericidins. The weight, however, is immediately taken from this argument by quoting the case of the dog, which shows a marked immunity to Anthrax, and yet does not possess in its serum any substances which are bactericidal for the anthrax bacillus.

The next step was the discovery by *R. Pfeiffer* of the Bacteriolysins. The name "Bacteriolysins" is often used as if it were synonymous with Bactericidins, but this is erroneous. A serum may kill bacteria without lysing them in the typical way about to be described, and, conversely, a bacterium may be "lysed" without necessarily being killed.

Pfeiffer injected living Cholera vibrios into the peritoneum of a guinea-pig which had been immunised against Cholera, and examined drops of the resulting peritoneal exudate by drawing it off with capillary pipettes and watching it microscopically in "hanging drop" preparations. As a control, he injected a similar quantity of Cholera vibrios into the peritoneal cavity of a normal guinea-pig. In the case of the normal animal the vibrios retain their shape and motility and multiply very rapidly, whilst, in the case of the immunised guinea-pig, the vibrios begin by losing their active motility and then soon become transformed into clear, granular bodies, which eventually disappear altogether. The peritoneal exudate is then found to be sterile—it gives no growth on culture media.

Metchnikoff showed that the same phenomenon could be produced in a test-tube, if one mixed Cholera vibrios with Cholera immune serum and added to this a small quantity of fresh peritoneal exudate of a normal animal.

Bordet showed further that by using immune serum, Cholera vibrios and a small quantity of the *fresh serum* of a normal animal, the same phenomenon is brought about.

To *Bordet* we owe the elucidation of the action of the substances concerned in this process of Bacteriolysis. His views, which are now generally accepted, are briefly as follows: when an animal is immunised against a certain bacterium (*e.g.* the guinea-pig against Cholera), specific antibodies are formed in the blood. Bordet calls these antibodies "sensitising substances" ("substances sensibilisatrices"), Ehrlich calls them "amboceptors" (for the reason explained, page 176).

These substances are *thermostable*—they resist heating at 55° C. for one hour. They are *specific*, that is to say they act only on the particular kind of bacteria which, when injected into an animal, have given rise to their production. Thus the bacteriolysins of an animal which has been immunised against Cholera would not lyse Typhoid bacilli.

They have the *power of fixing themselves to the bacteria*; if bacteria are left for some time in contact with the corresponding immune serum (which must be previously inactivated by heating to 56° C. for one hour), and are then centrifugalised to the bottom, the supernatant fluid will be found to have lost its amboceptors.

Bordet showed that the amboceptor alone cannot produce bacteriolysis ; this requires the co-operation of another substance, called by Bordet " alexine " and by Ehrlich " complement." This substance is *thermolabile*—it is destroyed by heating for half an hour at 56° C. It is *not specific*, is present in the blood of non-immunised animals and is *not increased during the process of immunisation.*

When Metchnikoff and Bordet did their early experiments in bacteriolysis, they were working with an old Cholera serum, the complement of which had been destroyed by age, and which therefore gave no bacteriolysis " in vitro " until some fresh serum or peritoneal exudate (complement-containing fluid) had been added. If, however, immune serum is used while still quite fresh, bacteriolysis occurs without the addition of any external complement.

This process of bacteriolysis is not an occurrence to which all kinds of bacteria are liable ; it is limited chiefly to Cholera vibrios and the Typhoid and Paratyphoid groups of bacilli.

Metchnikoff did not at first regard this Pfeiffer experiment with favour ; it seemed to detract from the importance of the phagocytes. On further experiment, however, he found that when Cholera vibrios are injected into the guinea-pig peritoneum, the leucocytes, normally present there, immediately disappear. Metchnikoff supposes that the leucocytes are injured and destroyed by the influence of the bacteria, and that, in the process of their destruction, substances are set free which cause the bacteriolytic changes in the vibrios. This destruction of the phagocytes he terms "Phagolysis." If this phagolysis is prevented, by certain simple procedures which he describes, no extracellular transformation of the bacteria occurs, but they are ingested by the phagocytes in their normal condition.

This point is unsettled even at the present day. Pfeiffer and many of his supporters, who have repeated Metchnikoff's experiments, have got quite different results—having always found extracellular lysis of the bacteria.

The Pfeiffer experiment may be employed practically for two diagnostic purposes :

I. To test an immune serum for the presence of bacteriolysins, and

II. To identify bacteria.[1]

I. If a serum shows a bacteriolytic power, for a certain bacterium, greatly increased as compared with that of the normal serum, the conclusion is infection with the particular bacterium.

For example, to prove that a person, supposed to be convalescent from Cholera, has really had this disease, the Pfeiffer experiment may be performed with the serum of the patient and known Cholera vibrios.

II. It may at times be very important to identify the true Cholera vibrio from other similar vibrios, and this may be done by the same experiment. For example, if some cases of suspected Cholera appear in a port and one fears a resulting epidemic, the vibrios isolated from the stools are tested by mixing them with a known Cholera-immune serum, and injecting the mixture into the peritoneal cavity of a guinea-pig.[1]

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[1] For the technique, see page 126.

MECHANISM OF THE BACTERIOLYTIC PROCESS.

Bordet's view is that the " substance sensibilisatrice " acts in bacteriolysis as a mordant does in the process of dyeing—it prepares the bacterium for the action of the " alexine," which then is able to bring about the lytic transformation. As in dyeing, different mordants are required for different materials, so different sensitisers are required for different bacteria. The alexine, however, is the same for all.

Ehrlich's view is quite different. He regards the immune body ("substance sensibilisatrice" of Bordet) as an amboceptor, *i.e.* a substance whose molecules have two chemical groups—a cytophile group, which unites with the bacterium, and a complementophile group, which unites with the complement. (See Ehrlich's Theory, page 176.)

It must, however, be remembered that this is altogether theoretical, and that there is no proof of the existence of such chemical groups.

5. COMPLEMENT.

What Complement is and how it was discovered, will have been gathered from the foregoing remarks on bacteriolysis.

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[1] In Germany official immune Cholera serum may be obtained gratis for the purpose.

It is a substance, present in blood serum and other body fluids, which unites with an antigen in the presence of the amboceptor corresponding to that antigen.[1] In the case of certain bacteria and other cells the result of this union may be obvious—there may be a lysis of the cell which is easily seen microscopically or with the naked eye. In many cases, however, this union of complement with the complex antigen-amboceptor gives no such evidence of its occurrence ; there are amboceptors which produce no lysis, but which still form a medium for the fixation of complement. In these cases the mixture must be tested for the presence or absence of free complement. ("Complement-fixation experiment," see page 126.)

The complement is easily destroyed by age, beginning to deteriorate within the first twenty-four hours. It is also easily injured by heat, and is completely destroyed by keeping at a temperature of 56° C. for half an hour. (A serum is said to be "inactivated" when its complement is thus destroyed.)

The complement, unlike the amboceptors, is not specific—the same complement will unite with any kind of antigen-amboceptor complex.

It is present in normal as in immune serum, and is not increased by processes of immunisation.

Complement may be separated from the amboceptor in a serum by filtration through sacs of collodion ; the amboceptors pass through, the complement does not. Furthermore, the union of antigen and amboceptor will take place at 0° C., whereas the fixation of complement will not take place at this temperature. By this means also the complement may be separated from the amboceptor in a serum.

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[1] See Glossary.

It has recently been shown by Ferrata that the complement consists of two parts which may be separated by dialysis; these are called the middle portion ("Mittelstück"), and the end portion ("Endstück"). The Mittelstück passes through the dialysing membrane, while the Endstück remains behind. Neither of these alone is able to produce lysis of sensitised blood corpuscles, but if first the one portion and then the other is added, the usual effect of the complement is obtained.

6. HAEMOLYSINS.

These are antibodies which "lyse" or "lake" red blood corpuscles—that is, alter their envelope and allow the haemoglobin to escape.

It occurred to Bordet that if immunisation against bacteria produces agglutinins and bacteriolytic substances in the blood, the injection of red blood corpuscles might give rise to the formation of similar agglutinative and lytic substances directed against the corpuscles. To find if this were the case, he injected the red blood cells of one animal into the peritoneum of another of a different species. By injecting, for example, rabbit's blood into a guinea-pig, he obtained in the guinea-pig's serum a substance which strongly agglutinated the rabbit's corpuscles, and also a substance which lysed them—a substance which proved quite analogous to the bacteriolysin just described. The lysis of the red corpuscles is evident to the naked eye, and this fact gives the process an important place in experimental research work, as will be seen later on.

The opaque, cloudy fluid containing the corpuscles clears up and becomes transparent, at the same time becoming stained by the haemoglobin which escapes from the corpuscles in the process of their dissolution.

The Haemolysins, like the Bacteriolysins, are amboceptors—they resist temperatures of 56° C. without injury, and they require for their action the co-operation of complement. They are also specific; the haemolytic serum lyses only corpuscles from the species of animal against whose blood it has been immunised and does not lyse corpuscles from another species.

It has already been pointed out that in normal serum there are present, in small amount, agglutinins, antitoxins and precipitins; the same holds good for the haemolysins, small quantities of which are found even in normal serum.

As will be seen later on, this fact is made use of in the Bauer-Hecht modification of the Wassermann reaction, where, for the purpose of simplifying the experiment, advantage is taken of the normal haemolysins in human blood, which are sufficient to produce lysis of a small quantity of sheep's corpuscles.

To the student of Immunity it will at first sight appear as if these haemolysins occupied an unduly prominent place. The reason for this is that the process of haemolysis is so easily observed—requiring only test-tube experiments and naked-eye examinations—and has on that account come to be used in a great variety of experiments for the elucidation of the qualities of immune bodies and their relations to complement, etc. Then the haemolytic experiment came to be used as a test for free complement in various mixtures, and as such it has established itself in the complement-fixation method of Bordet and Gengou, which will be considered in a later chapter.

7. OPSONINS.

In 1903 *Wright and Douglas* announced the discovery of a new anti-bacterial substance in the blood serum. To this substance they ascribed the function of preparing bacteria for ingestion by the phagocytes, and, therefore, named it "Opsonin" (from *Opsono* = I cater for; I prepare food for).

DEFINITION.

The *opsonin* is something which acts on bacteria in such a way as to render them more easily ingested by the leucocytes—more phagocytable.

HISTORY.

Long before this date, however, there had been much work done in the research of substances, in blood serum, which facilitate phagocytosis. *Metchnikoff*, who had discovered the process of phagocytosis, and who regarded it as the primary factor in Immunity, as early as 1893 expressed the opinion that serum might contain certain ingredients which had the property of stimulating the phagocytes; for these he proposed the name of "Stimulins."

Whether such substances actually exist is, to this day, a debatable question.

Denys and Leclef, however, were the first (1895) to prove that the serum of an immune animal has the property of aiding phagocytosis, and that this property is quite distinct from any bactericidal or other known effect of such serum. They opposed the theory of "stimulins," and showed that the leucocytes are not directly affected by the serum, but that the serum acts on the bacteria and, in some way, renders them more liable to phagocytosis. They suggested that the serum might act by neutralising the endo- and exotoxins of the bacteria, and so depriving them of their defensive weapons against the phagocytes. Numerous other workers had occupied themselves with this question—Marchand, Mennes, Sawtschenko and Levaditi had all made important contributions—but it still remained in a rather chaotic condition when, in 1903, Wright and Douglas made their first publication on "the rôle of the blood fluids in connection with phagocytosis." The first problem which they dealt with was the question of whether, as Metchnikoff had supposed, the blood fluids aid phagocytosis by exerting a direct, stimulating effect upon the phagocytes, or whether, as suggested by Denys and Leclef, they acted by effecting a modification in the bacteria.

In their experiments they used a modification of the technique devised by Leishman for measuring the phagocytic power of blood. In this method, equal volumes of a bacterial suspension and of blood are measured off in a capillary pipette, then blown out on a slide and mixed. A drop of the mixture is then covered with a cover-glass and the preparation put in the incubator at 37° C. for 15 minutes. At the end of this time a film preparation is made of it by drawing the slide and cover-glass gently apart. It is then coloured by Leishman's stain and examined.

Wright and Douglas modified this method (1) by conducting the phagocytosis in capillary tubes instead of on a glass slide, and (2) by decalcifying the blood with sodium citrate to avoid coagulation, and making it possible to get the leucocytes separated by centrifugalisation. (For full technique, see page 119.)

Separate experiments were done to test the effect of blood serum, (*a*) on the bacteria, and (*b*) on the phagocytes. The conclusion arrived at was that "the blood fluids modify the bacteria in a manner which renders them a ready prey to the phagocytes." This is spoken of as an "opsonic effect" of these fluids, and the substances in the fluids which produce the effect are termed "Opsonins."

The question as to whether the serum also contains elements which directly stimulate the phagocytes was left unsolved. Wright's results were soon confirmed by the work of *Bulloch and Atkin*, *Hektoen and Ruediger* and others, and the opsonic effect of blood serum was accepted as an established fact in Immunity.

PROPERTIES OF OPSONIN.

The opsonin is characterised by great instability. It disappears gradually from serum on standing, and, even in the dark, it is reduced to about one-half after the lapse of five or six days. It is also thermolabile, being injured by temperatures over 50° C., and heating at 60° C. for ten minutes destroys it completely.

In subsequent papers, Wright and his collaborators went on to show the wide clinical significance of these discoveries, and so, quite apart from their purely scientific aspect, the " opsonins " attracted very much interest, especially in England. Wright showed that patients suffering from acne, sycosis or boils had invariably a defective opsonic power of their blood against the causal micro-organism. He further showed that these patients could be successfully treated by vaccination with emulsions of dead cultures of the corresponding bacteria, and that, coincidently with the clinical improvement, there was a rise in the opsonic power of the patient's serum against these bacteria.

As far as could be made out by these observers, the leucocytes from the patient did not differ in their phagocytic capacity from ordinary leucocytes.

Wright also claimed that the opsonin which is present in the blood of a vaccinated patient, and which has been increased in amount by the inoculation processes, does not differ in respect of its resistance to heat and sunlight, from the opsonin found in normal serum.

Experiments were conducted " in vitro " with the new " opsonic index " technique, in order to find if all pathogenic bacteria are affected by these opsonins. It was found that the opsonic action of the blood fluids is exerted on Staphylococcus, Bac. Tuberculosis, Bac. Pestis, Micrococcus Melitensis, Bac. Dysentericus (Shiga), Bac. Coli, Pneumococcus, Streptococcus, Bac. Anthracis, Bac. Typhosus and Vibrio Cholerae.

Bac. Diphtheriae and Bac. Xerosis, however, seemed to be exceptions, and were not affected by the opsonins.

Other workers have not come to quite the same conclusions regarding the phagocytability of various micro-organisms. It is, as will be mentioned later, largely a question of virulence, a freshly-isolated virulent bacterium being frequently unphagocytable, whereas the same bacterium, after going through several sub-cultures in the laboratory, may be phagocyted with the greatest facility.

RELATIONS BETWEEN OPSONINS AND THE PREVIOUSLY KNOWN ELEMENTS OF THE SERUM.

Wright claims that the opsonins are newly discovered substances, quite distinct from the already known antibodies, such as complement and amboceptors.

On this ground he is opposed by many leading authorities. Especially on the question of the opsonins in immune serum, his views have met with much contradiction, a great many workers finding that immune opsonins resist heating to 60° C. (are "thermostable"), while Wright holds that all opsonins, normal and immune, are destroyed at this temperature (are "thermolabile.").

Neufeld and Rimpau, in experiments with streptococci and antistreptococcic serum, found that the immune serum opsonises just as well after inactivation at 59° C. They therefore propose to separate the phagocytosis-promoting substances found in immune serum (which they call " Bacteriotropins ") from those found in normal serum (" Opsonins ").

Hektoen finds similarly that the immune opsonin, in a convalescent Typhoid case, resists, at least in part, heating for half an hour at 70° C.

Dean also finds that the opsonic substances in immune serum resist heating at 60° C.

Muttermilch found that immune opsonin can filter through sacs of collodion, in this respect also resembling the amboceptors already known.

Many authorities hold that immune opsonin is identical with amboceptor. If the immune serum is undiluted, the amboceptor is able to produce the opsonic effect without the aid of complement; if, however, the (inactivated) immune serum is much diluted, it requires the aid of some added complement in order to exercise its opsonic effect. The question, however, cannot yet be regarded as definitely solved.

On the other hand, most authorities are now agreed that normal opsonin is identical with complement.

The experiments of Neufeld and Hühne, Muir and Martin, Becher, Levaditi and Inman, Bordet and others all support this contention.

These and many other experiments may be quoted in support of the view that opsonins are not to be separated from the complement of a normal serum.

For this reason Levaditi suggests that it would be well, meanwhile, to speak of the "opsonic powers" of the fresh blood serum and not of its content in "opsonins," as, so far, there is not sufficient ground for the belief that such substances exist as an individual and separate element of normal serum.

It may be that normal amboceptors also play a part in the opsonic process, but this question cannot be entered into here.

Normal opsonin, like complement, is destroyed by heating for ten minutes at 60° C.

Levaditi has shown that the normal opsonin is absorbed when fresh serum is left in contact with bacteria or with various organ cells (*e.g.* liver cells), exactly as complement in similar circumstances is absorbed.

Muttermilch showed that normal opsonin does not filter through collodion sacs, in this respect also resembling complement.

Muir and Martin found that "a bacterium, combined with the homologous immune-body, absorbs or fixes bactericidal complement, haemolytic complement, and normal opsonin alike."

Ferrata has recently shown that by dialysis of a serum the complement may be separated into two portions—a middle portion ("Mittelstück"), which passes through, and an end portion ("Endstück"), which remains behind. Neither of these is alone able to cause haemolysis of sensitised blood corpuscles, but the union of the two portions produces this result.

Precisely the same occurs with the opsonin of a normal serum—by dialysis it is separated into two portions which are ineffective apart, but active again when united.

CLINICAL IMPORTANCE OF OPSONINS.

On this question also opinions are much divided. Wright claims that the estimation of the opsonic power of a patient's serum may be of value, (*a*) as an aid in diagnosis, and (*b*) as a guide in vaccine treatment. He states, for example, that in patients suffering from boils, sycosis, etc.—staphylococcic infections—the opsonic power against staphylococcus is invariably low.

By treating the patient with a vaccine of the corresponding bacteria, the patient may be cured of these conditions, and, coincidently with the clinical improvement, there is an increase of the opsonic power of the patient's serum.

This principle has been applied by Wright and his followers to the diagnosis and treatment of the most diverse infections — Tuberculosis, localised Staphylococcic infections, localised Streptococcal infections, Pneumonia, Pyaemia, Ulcerative Endocarditis, Bronchitis, Coryza, etc., etc. In the early days opsonic estimations were held to be essential to successful vaccine therapy ; now experience has fixed the dosage for many cases, and the opsonic estimations are very generally dispensed with. The question of Vaccines is dealt with elsewhere (page 106).

With regard to opsonic estimations, it is difficult to argue scientifically that they really possess the value that has been claimed for them.

In testing the opsonic index of a patient's serum, several things are taken for granted, which are by no means proved :

1. It is assumed that the bacteria in the patient's body behave towards the opsonins and the phagocytes in the same way as the bacteria used in the experiment "in vitro." This is an untenable hypothesis ; it has been repeatedly proved that, under many circumstances, bacteria become resistant to phagocytosis and will not allow themselves to be ingested by the leucocytes even in presence of serum. This is especially the case with highly virulent bacteria, such as those in question in the various diseases under investigation. For example, Pneumococci freshly isolated from a case of pneumonia may entirely resist phagocytosis. They, however, soon become phagocytable when cultivated on ordinary artificial media. An opsonic estimation, therefore, done with a laboratory culture of indefinite age and virulence, can hardly give any criterion of the power of the patient to overcome the "resistant," fortified pneumococcus, with which he has to deal.

2. It is assumed that the leucocytes are a constant factor in the calculation, that in the infection under consideration the leucocytes need not be taken into account. This, as has been shown by Rosenow and others, is erroneous. Rosenow has proved, for example, that the leucocytes in pneumonia differ from normal leucocytes both in their power of ingesting pneumococci and of destroying them after they are ingested.

3. It is assumed that phagocytosis plays a preponderant part in overcoming the infectious disease under investigation. In many cases, however, this is by no means proved. For example, in Tuberculosis, it is not the polymorphonuclear leucocyte but the lymphocyte which seems to have the important rôle, and hence it is difficult to see how an estimation of the opsonic index can elucidate the question of diagnosis or treatment.

CHAPTER III.

THE BODY CELL AS A FACTOR IN IMMUNITY.

PHAGOCYTOSIS.

"IF ever there was a romantic chapter in Pathology, it has surely been that of the story of Phagocytosis." So said the late Lord Lister in his Presidential Address to the British Association in 1896, and so may we, with equal fervour, say to-day when many more pages of the story have been turned over.

The author of the "story" is Élie Metchnikoff; to his genius and untiring zeal we owe practically every line. Others, it is true, have come in and attempted to change the plot and to erase parts here and there, but the most important effect of these interruptions has been that they incited Metchnikoff to write over again, with a bolder pen, the pages they attempted to blur. To-day the position of the Phagocytosis Theory is assured, and whether we talk of opsonins and bacteriotropins or of aggressins and "resistant races" of bacteria, we are all the time admitting that the important factor is ultimately the phagocyte.

The term "phagocyte" was coined by Metchnikoff to designate the amoeboid cells capable of seizing and digesting micro-organisms, red blood corpuscles and other formed elements; the process he termed "phagocytosis."

Before Metchnikoff commenced his studies, several observers had noted that leucocytes might assist in the destruction of micro-organisms.

Panum (1874) suggested that the bacilli of putrefaction might disappear from the body by making their way into the blood corpuscles and being carried off to the spleen, etc.

Roser (1881) also noted the capacity of certain "contractile cells" to ingest "the enemy which enters the animal body."

These statements, however, were supported by no scientific data or subsequent research, and were the merest speculations. When Metchnikoff commenced his work, he had not even heard of them.

It was through his zoological researches that Metchnikoff was led to the discovery of the part played by phagocytosis in Immunity. He had shown that the simplest unicellular organisms—*e.g.* the amoeba—feed themselves by this process. When a nutrient particle arrives in the vicinity of an amoeba, the latter, by reason of its "irritability," becomes incited to send out processes of its protoplasm (pseudopodia), which flow around the particle and ultimately gather it into the interior of the cell. The particle then, as Metchnikoff was able to show, undergoes a process of intracellular digestion and disappears within the protoplasm of its host.

Metchnikoff traced this same process of nutrition through many unicellular animals, then through actinians, sponges and certain pelagic animals of simple organisation.

At this epoch in his career, he chanced to listen to the reading of Cohnheim's treatise on General Pathology, and was greatly impressed by the part dealing with the phenomena of inflammation. The account of the diapedesis of the leucocytes through the walls of the blood vessels struck him as being very significant. He resolved to make a comparative study of the phenomena of inflammation in the lower animals, believing that the processes there, which were much more simple to watch, might be an index to the processes occurring in the higher vertebrates.

He began by observing the large bipinnaria larvae of star-fish which, owing to their transparency, were admirably suited for the purpose. He inserted rose prickles into their bodies, and found that these prickles were soon enveloped in a mass of amoeboid cells. From this he concluded that, in inflammation, the exudation must probably be regarded as a reaction against any sort of injury whether mechanical or due to bacterial invasion.

Up to this time, Pathologists had regarded the bacteria as attacking the white corpuscles, and sometimes gaining their way into the interior of these cells by forcing a passage. It was, therefore, quite a novel suggestion that the phagocytes might be the pursuers.

Metchnikoff now endeavoured to connect his various discoveries and to formulate the idea that " the intracellular digestion of unicellular organisms and of many invertebrates had been hereditarily transmitted to the higher animals, and retained in them by the amoeboid cells of mesodermic origin. These cells, being capable of ingesting and digesting all kinds of histological elements, may apply the same power to the destruction of micro-organisms."

In his subsequent experiments, he again followed the method of Comparative Pathology, and traced the defensive reaction against an invading micro-organism, from small transparent invertebrates up to man. In this way he demonstrated clearly the uniformity of the reaction.

The student may gain an exact idea of the processes of phagocytosis and intracellular digestion by the simple experiment of injecting some nucleated red blood corpuscles (*e.g.* defibrinated goose blood) into the peritoneal cavity of a guinea-pig. Small quantities of the peritoneal exudate are then drawn off in capillary pipettes and " hanging drop " preparations of this examined microscopically. The red corpuscles will be seen in the interior of large mononuclear leucocytes (macrophages). They lose their haemoglobin and become more and more irregular in outline; finally all except the nucleus disappears. Thus a process of intracellular digestion is seen here as in the amoeba.

In the phenomena of inflammation as seen, for example, in the subcutaneous tissue of man, we have the same process. As soon as the infective agent penetrates into the tissue, diapedesis of the white blood corpuscles occurs. These white corpuscles now wage war on the intruding micro-organisms, seize them by sending out protoplasmic processes, and convey them into the cell interior, where they undergo a process of digestion.

This leucocytic reaction is set up not only by the advent of bacteria, but by the introduction of all kinds of foreign elements, and even sterile fluids.

According to Metchnikoff, the bacteria are digested within the leucocytes by means of ferments secreted by the leucocytes; these ferments he names "cytases," and he holds that they are the same substances as those designated "complements" by Ehrlich or "alexines" by Bordet.

Metchnikoff has studied very exhaustively the rôle of phagocytosis in Natural and in Acquired Immunity.

In Natural Immunity the resisting power enjoyed by the immune animal seems to be the direct result of an efficient phagocytic capacity. This subject has been worked out in very many different species of animals. One experiment of Bordet may be cited as an instructive example:

Guinea-pigs possess a Natural Immunity against streptococcus pyogenes. This Immunity is not absolute, as the animal may succumb if very large doses are injected or if the strain of streptococcus is specially virulent. Bordet found that the inoculation of an emulsion of streptococci into the peritoneal cavity of the guinea-pig gave rise to an afflux of leucocytes, which seized the cocci and destroyed them. If the culture is very virulent, or the dose is too large, the phagocytosis is incomplete; some streptococci escape and begin to multiply. This new generation, however, is a "resistant race," and does not allow itself to be phagocyted—the resistant streptococci form capsules, which seem to protect them from phagocytosis.

Natural Immunity, therefore, is evidently only a question of degree, and the degree in each case depends on the possibility of efficient and complete phagocytosis.

The part played by phagocytosis in Acquired Immunity has been a much contested question; something has already been said of this in Chap. I. (page 6). Suffice it to say here that the phagocyte alone has maintained its position through all the controversies. The importance of antitoxins, bactericidins, bacteriolysins, opsonins, etc., may wax and wane, but the importance of the phagocyte remains undisturbed.

In all the controversies as to whether "stimulins" may help to make the phagocyte more active, whether "opsonins" and "bacteriotropins" may make the microbe more "savoury," and whether aggressins and bacterial capsules may make it more "unsavoury," the tacit admission remains that the phagocyte is king.

THE PHAGOCYTES.

In order to make our nomenclature clear, a few preliminary remarks on the classification of leucocytes are necessary.

Metchnikoff divides the Phagocytes into two great classes: (*a*) Macrophages, and (*b*) Microphages.

(*a*) The group of *Macrophages* includes large amoeboid cells of the spleen pulp and of the lymphatic glands and omentum, certain endothelial cells and perhaps some cells of the connective tissue. The so-called "Giant cells" are simply polynuclear macrophages. These macrophages may be either "fixed" cells, stationary in the tissues, or they may be free, amoeboid cells, or "wandering cells," which, owing to their lack of attachment, are able to migrate from place to place.

General Characters. The macrophages are large cells with large nucleus (which is usually single but is sometimes multiple, as in "giant cells"), and protoplasm, which is slightly basophile and devoid of granules.

(*b*) The group of *Microphages* contains the polymorphonuclear leucocytes.

The distinguishing characteristics of these are the lobed nucleus and the neutrophile granulations of the protoplasm.

It is this cell which is multiplied in the body in leucocytosis. It arises from the polynuclear myelocyte of the bone marrow. (The eosinophile leucocytes have also been shown to ingest bacteria, but the important cell for phagocytic purposes is the neutrophile.)

The microphages all belong to the group of "free" phagocytes.

Besides being distinguished morphologically, the macrophages are separated from the microphages by functional characteristics. In a general way it may be said that the macrophages manifest their phagocytic activity chiefly against cells and cell débris, whilst the microphages deal with bacteria. There are, however, exceptions to this rule: for example, the giant cells, which are so prominent in the phagocytosis of Tubercle Bacilli and Actinomyces, are macrophages.

It will easily be understood that the "wandering cells" are of much more importance than the fixed phagocytes, as, by their motility, they are able to make their way hastily to any menaced point of the organism.

LEUCOCYTOSIS.

The normal number of white corpuscles in the blood of an adult man is 7000 to 8000 per c.m.m. Of these, about 35 per cent. are lymphocytes and about 62 per cent. are polymorphonuclear leucocytes.

In the course of certain infectious diseases, the number of the leucocytes may be greatly augmented. This augmentation usually affects the polymorphonuclear neutrophiles—and this is easily understood, as these are the "microphages."

For example, in a case of Pneumonia, the polymorphonuclear neutrophile count may go up from 4200 to over 18,000 per c.m.m.

In a few cases the augmentation has been found to occur in the number of eosinophile cells; this is the case in certain skin diseases (pemphigus, etc.), and also in some diseases due to animal parasites (Trichiniasis, Ankylostomiasis, etc.).

LEUCOPENIA.

In certain conditions the total number of leucocytes in the blood may be demonstrably diminished;

this is known as "Leucopenia." An example of this is seen in Typhoid Fever.

The Process of Phagocytosis and its Mechanism in the Light of Recent Discoveries.

It becomes daily more obvious that in order to understand this phenomenon of phagocytosis it is futile to focus one's attention entirely on the leucocyte or entirely on the serum as has previously been done; all three factors concerned in the process—phagocyte, serum and bacteria—must be kept in view.

1. *The Phagocyte.* It was originally supposed that the phagocytes, alone and unaided, were able to rid the body of invading bacteria. It is now known that, without the co-operation of the body fluids, their power is extremely limited. Bacteria can, to a slight extent, be ingested by phagocytes in a medium of salt solution—therefore without any intervention of the body fluids. This so-called "spontaneous phagocytosis," however, occurs only to a very limited degree, and only with avirulent bacteria.

The phagocytes, on the other hand, put forth a special effort in view of invasion. Leucocytosis, as is known, plays an important part in many infective diseases. Here we have to deal with a *numerical* addition to the defensive forces. It is probable that the leucocytes also undergo *qualitative* changes which increase their anti-bacterial power. It had been held by the majority of researchers that the leucocytes remained unchanged during the process of immunisation of an animal—that "immune" leucocytes were identical with normal leucocytes. Recent observations of Rosenow and others have, however, made this conclusion very doubtful. Rosenow finds, for example, that leucocytes from a patient during an attack of pneumonia are different from normal leucocytes in their increased power, both of ingesting bacteria and of destroying them after ingestion.

It is thus probable that, qualitatively as well as quantitatively, the leucocytes may undergo "adaptation" during the process of immunisation.

2. *The Serum.* There can be no doubt, at the present day, as to the importance of the body fluids in phagocytosis. It is obvious from many experiments of Wright and others that the process is much facilitated by the presence of serum—in fact, that "spontaneous phagocytosis," or phagocytosis in salt solution, takes place only to the most limited extent.

3. *The Bacteria.* It is certain that the bacteria fortify themselves against phagocytosis in many cases, where they get sufficient time. If the phagocytes are able to ingest all the invading bacteria immediately, there is no time for these to strengthen themselves against attack. If, however, some bacteria escape immediate phagocytosis, these set to work to "vaccinate" or "immunise" themselves against the phagocytes. In the following chapter (page 70) this is fully treated; here may be instanced the formation of a capsule by certain bacteria as a means of hindering phagocytosis.

According to Bail, the bacteria have further the power of secreting substances which paralyse the leucocytes and thus arrest phagocytosis. These substances are called " Aggressins."

Levaditi has made a most careful study of the different stages of the process of phagocytosis, and has been able to define them very clearly. By using Trypanosomes of Nagana and the serum of a guinea-pig immunised against this protozoon, he has been able to follow each phase of the phenomenon more closely than had been done before. The Trypanosomes, owing to their large size and their very vigorous movements, are most easily observed.

Trypanosomes and leucocytes are mixed with some immune serum (antitrypanosome serum), and the result is observed microscopically. The Trypanosomes, which are at first swimming about freely in the fluid, become attached by their non-flagellated end to the leucocytes, and then make vigorous movements of the flagellated end in an apparent attempt to get free. The leucocytes may soon be seen to put forth amoeboid processes, which gradually surround the attached end of the Trypanosome. Very active movements of the Trypanosome go on until the nucleus is phagocyted, and give to the process quite the dramatic appearance of a deathly struggle. The entire Trypanosome becomes transparent and rounded, and is eventually completely enclosed within the protoplasm of the leucocyte.

The phagocytosis does not occur if, instead of immune serum, one employs normal serum (likewise heated to 56° C.). It is, therefore, evident that this antitrypanosome serum contains immune opsonins.

The phenomena, according to Levaditi, can be divided into two phases :

1st. The phase of Fixation, and
2nd. The phase of Ingestion.

1st. *The Phase of Fixation of the Phagocytable Object to the Leucocyte.* Bacteria, which have been sensitised (opsonised) by immune serum, fix themselves to the protoplasm of the leucocytes. There is evidently a special affinity between the sensitised antigen and the leucocytic protoplasm. For this phase it matters not whether the leucocytes are alive or dead ; the fixation is evidently a physico-chemical phenomenon, which does not require the vital activity of the leucocytes.

Rosenow has shown this phenomenon of fixation with pneumococci in presence of immune pneumonic serum ; the pneumococci are seen in circles around the leucocytes and evidently touching them. If dead leucocytes are used, the first phase takes place, but no ingestion of the pneumococci occurs.

2nd. *The Phase of Ingestion of the Micro-organisms.* This is a vital phenomenon—it only occurs if the leucocytes are alive. If the leucocytes are injured or are cooled to 0° C., Levaditi has shown that no ingestion takes place.

It seems, too, as if the leucocytes were stimulated by the fixation of the micro-organism to secrete a microbicidal substance, in order to destroy it. Experiments of Bordet on the phagocytosis of Bac. Anthracis support this view.

It should be remembered that highly virulent races and "antibody-resistant" races of micro-organisms do not allow themselves to be phagocyted even in presence of immune serum.

CHAPTER IV.

THE MICRO-ORGANISMS AS A FACTOR IN IMMUNITY.

A. RESISTANT RACES OF BACTERIA.

In this chapter the point of view is changed. In what has gone before, consideration has been directed exclusively to the defensive armoury of the animal body against the micro-organism, and in the spectacle of all the appalling big guns which are brought forth for the destruction of a tiny bacterium, one finds that there is nothing very heroic! It is now, however, necessary to consider the defensive measures adopted by the bacterium in its efforts for self-preservation, and it soon becomes evident that it does not take things "lying down"—that it does not meekly resign itself to being swallowed by a phagocyte or weakly permit itself to be shackled by a grim amboceptor which links it on to a complement, which thereupon proceeds to rend it to pieces! It will be found that the microbe is made of "sterner stuff." The genesis of a "resistant race" of *Trypanosomes* may be given as an example: if Trypanosomes of Nagana are injected into the peritoneum of a guinea-pig, they appear in the blood on the second day after injection; they then multiply enormously till the fifth or sixth day, when the animal has a " crisis " and the Trypanosomes disappear from the circulation. After a few days, however, Trypanosomes again show themselves in the blood stream, but these Trypanosomes are a new race—they have acquired properties which distinguish them very markedly from the Trypanosomes present before the crisis. If brought in contact with an immune serum, which would cause lysis of the original Trypanosomes, they retain their form and seem unaffected—they have become " antibody-resistant." They have also become " phagocytosis-resistant "—they do not attach themselves to leucocytes, and do not allow themselves to be phagocyted. They are therefore designated a " resistant race " of Trypanosomes. They probably represent a race which is the result of natural selection and the survival of the fittest—all the ordinary Trypanosomes are killed off by the antibodies formed in the blood of the guinea-pig, and only a few specially resistant individuals survive ; from these a new race originates.

The same occurs in *Relapsing Fever*, where the spirillum succeeds in vaccinating itself and producing the fresh paroxysms, during which it reappears in the blood, despite all the defensive measures of the body to expel it.

In *Syphilis* there is evidently an example of the same phenomenon. As has long been known, persons who show the primary symptoms attain a marked immunity to a re-infection. This immunity, however, does not apply against the spirochaetes which have already established themselves in various foci in the body, and which are liable to go on for years causing recrudescences of the disease. The body of the syphilitic has acquired immunising properties which are sufficient to protect it against the original spirochaete, but meanwhile the spirochaete in the body has been vaccinating itself and is fortified against all the new mechanism brought into play against it.

The production of resistant races has been very evident in the work which has been carried on for years by Ehrlich and his pupils, with regard to the action of various arsenical compounds on protozoa —work which has culminated in the brilliant discovery of "606." When experimenting, for example, with Trypanosomes and Atoxyl, they found that where all the micro-organisms were not killed off, those that escaped acquired a new power of resistance to the poison—they became atoxyl-resistant.

It is easily understood that this factor must add greatly to the difficulty of treatment of every infection where it occurs.

The fact that bacteria, in certain cases, fortify themselves against the immunising properties of the body has been known for some time. Bordet, for example, had shown that a *streptococcus* may, under certain conditions, protect itself by the formation of a capsule. These capsulated streptococci do not allow themselves to be phagocyted.

It has also been shown by Rosenow that *Pneumococci* fresh from a pneumonia patient resist phagocytosis. Only after repeated subculture on artificial media do they lose this resistant property, and permit themselves to be phagocyted. Eventually, when they have lost all their virulence, they become so easily phagocytable that the leucocytes may take them up in a medium of salt solution without the aid of serum.

Rosenow points out how effective this mechanism of resistance may be in enabling pneumococci to triumph over all the immunisation mechanism of the body. For example, in a case of pneumonia or pneumococcal endocarditis, the leucocyte count may show 10,000 per c.m.m., *i.e.* 10,000,000 per c.c.m. to take up, let us say, 100 cocci per c.c.m. (a fair average of the number present). This makes 100,000 leucocytes to each pneumococcus—and yet the patient dies !

It is known also that non-agglutinable (agglutinin-resistant) races of *B. Typhosus* may be obtained by cultivating the germ direct from the body of the patient. The first culture, and perhaps a few succeeding subcultures, may resist agglutination, but this resistance is soon lost on artificial media.

These agglutinin-resistant bacteria have been found by Leishman in post-typhoid abscesses, and it is probably such resistant races that are present in "Typhoid Carriers."

Similarly with Bac. Anthracis, it is found that whilst agar-grown cultures are freely phagocyted in presence of serum, the bacteria grown in the animal body are non-phagocytable. In their growth in the body they form a capsule, and it would appear to be the possession of this capsule which confers on them protection against the leucocytes.

Then in *Tuberculosis* it seems extremely probable that the same factor comes into play. Koch has shown that if the Bacillus Tuberculosis be injected subcutaneously into tuberculous guinea-pigs, there is an acute inflammatory reaction at the site of inoculation; a slough then forms and carries off with it great numbers of bacilli. The animal is obviously immune to reinfection, but nevertheless its original infection usually becomes generalised and causes miliary tuberculosis and death. Evidently then the Bacillus Tuberculosis within the body has developed powers of resistance which were entirely lacking in the bacilli as they originally infected the animal.

Such changes in the tubercle bacillus would explain how it may survive for long years in the human body, and eventually kill a patient who has indeed been able to immunise himself against the originally infecting bacillus, but not against this reinforced new race.

B. AGGRESSINS.

According to the theory of Bail, a micro-organism is pathogenic because, when brought into contact with the protective mechanism of the body, it is able to secrete substances which paralyse these protective agents, especially the leucocytes.

The substances thus formed by the bacteria in the living body, he calls "Aggressins." The production of these substances, according to Bail, is called forth by the necessity for the bacterium to protect itself against phagocytosis, etc.

In support of this theory, Bail was able to show that in the exudate found in rapidly fatal infections, substances are present which have the peculiarity of promoting an infection when inoculated into another animal along with the corresponding bacteria. A dose of the bacteria, which otherwise would not cause death, is made lethal by the addition of a small quantity of this fluid containing the so-called " Aggressins."

CHAPTER V.

IMMUNITY REACTIONS EMPLOYED FOR THERAPEUTIC ENDS.

A. SERUM THERAPY.

In serum therapeutics we find the most striking triumph and the most humiliating defeat of the clinical Immunisator. The few genuinely antitoxic sera represent the triumph, the many antibacterial sera represent the defeat or, rather one should say, mark the limitations of passive immunisation.

In vaccine treatment, as will be shown later, the patient is stimulated to produce his own protective substances; in serum therapy he receives protective substances which have been formed in the body of another animal. In the former case he is *actively* immunised; in the latter he is *passively* immunised.

When, in 1890, V. Behring and Kitasato made their brilliant discoveries in connection with Diphtheria and Tetanus toxin and antitoxin, boundless possibilities immediately presented themselves to those interested in the serum treatment of infectious disease. These possibilities have, up to a certain limit, been magnificently realised; beyond that limit there has been only disappointment and the invariable discovery of the scientist that Nature is never so simple as she pretends to be.

If all pathogenic bacteria acted like B. Diphtheriae and produced their harmful effects by the formation of a soluble toxin, then the problem of treatment would resolve itself into finding the most satisfactory method of producing effective quantities of antitoxin. A fever hospital would be fully equipped with a row of bottles of antitoxins against Typhoid Fever, Scarlet Fever, Measles, Whooping Cough, etc., and an adequate supply of syringes. But, alas! the bacteria are more subtle! All do not produce true toxins, and in many cases their mode of attack is still quite obscure. In the treatment of diseases due to bacteria which do produce true toxins, the method of combating them with antitoxic sera has been eminently successful.

A bacterial toxin strictly speaking (" true toxin ") is a secretion product of the bacterium which is given off into the medium in which it is growing. When injected into the animal body it gives rise to the production of antitoxin which neutralises the toxin.[1] The number of known pathogenic bacteria which produce such toxins is comparatively small; B. Diphtheriae and B. Tetani are the chief members of the group, but B. Botulinus and the Dysentery bacterium of Kruse-Shiga must also be included.

For the bacteria which cause the great majority of human infections no true toxins can be discovered, and therefore it is necessary to look elsewhere than to antitoxic sera for the treatment of such infections.

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[1] See "Antitoxins," page 15.

The antitoxic sera will be dealt with first.

I. ANTIDIPHTHERITIC SERUM.[1]

In the production of Antidiphtheritic Serum on a large scale, horses are used because of the large amount of serum they can yield and because horse serum is less toxic to man than is that of many other animals.

The toxin used for immunising the horses is prepared by growing Diphtheria bacilli in alkaline bouillon. After eight days' growth the bouillon contains quantities of toxin which vary with certain conditions. The bacteria are then allowed to settle to the bottom, or the bouillon is filtered in order to get the toxin free from bacteria.

To immunise the horse it is necessary to begin with a very small dose. Some horses rapidly yield a good serum ; others react much more slowly.

When the serum contains a satisfactory amount of antitoxin the animal may be bled to death, or it may be only partially bled and then allowed a rest and used once again.

The strength of the serum is estimated according to the method of Ehrlich ; the antitoxic power can then be set forth in the number of antitoxic units. This unit is universally used as a standard. It is not necessary to know how Ehrlich arrived at this unit, which he designates "I.E." (Immunitätseinheit), any more than it is necessary for us to know how the mile was originally fixed on as the standard of distance. An active serum may have 500-1000 units per c.c.m.; if it contains less than 200 units per c.c.m. it is discarded as too weak.

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[1] For the mode of production of antitoxic sera in general, see section on "Antitoxins," page 15.

In order to reduce the number of c.c.m. which it is necessary to inject into a patient, endeavours have been made to concentrate the serum. The globulin of the serum is precipitated by means of a solution of ammonium sulphate, and this precipitate carries down the antitoxin. The precipitate is then redissolved.

Employment of Antidiphtheritic Serum. Subcutaneous injection of the antitoxin is the method usually employed.

In very grave cases, where the serum is not given until very late, an intravenous injection has been recommended as offering more hope.

Dose. One need never fear giving too much; it is infinitely better that the dose be excessive than that it be insufficient. Many experienced practitioners recommend a quantity of 4000 units as the ordinary dose for a case of moderate severity. Some American physicians give much larger doses—10,000 to 100,000 units—and report good results, although in all probability such excessive doses are quite unnecessary.

The dosage is irrespective of age; children tolerate the serum even better than adults. Newly-born babies may quite safely receive the injections.

Above all, the injection should be made *early*—the earlier the injection the better the result, as all statistics show. In doubtful throat cases, where there is any suspicion of Diphtheria, the correct treatment is to inject at once and not to wait for a bacteriological report, if this means losing a day.

In children under five years, it is especially important to give antitoxin at once if there is any suspicion of Diphtheria, for in these cases the Diphtheria evolves rapidly and the mortality is very great, and, on the other hand, the risk of any ill effect from the serum is practically nil. In families where there is an outbreak of Diphtheria, it is wise to give preventive inoculations to very young children—especially those under two years—as with them Diphtheria is so often fatal that it is unwise to run the risk.

In two-thirds of the cases of Diphtheria, the bacteria disappear from the throat by the fifteenth day; in one-third of the cases, however, they persist. For the removal of these bacteria, which are a source of danger to the community, besides using gargles, etc., the patient should have pastilles of Antidiphtheritic serum which he allows to melt in his mouth (one every hour for five or six days).

With regard to preventive injections of Antidiphtheritic serum, the following recommendations made by the French Academy of Medicine are noteworthy:

1. Preventive injections of serum are capable of immunising children exposed to infection by Diphtheria. They have never caused any untoward results.
2. The injections are particularly indicated in families where a case of Diphtheria develops, for the protection of the other children.

3. Injections are likewise indicated in any agglomeration of children (school, crèche, hospital ward, etc.) in which a case of Diphtheria occurs.

The reduction in the mortality from Diphtheria since the introduction of antidiphtheritic serum has been very marked. In England, the statistics show a mortality of 9-10 per cent. in 1908, as compared with 30 per cent. in 1894. According to Loeffler's statistics, the mortality from Diphtheria in the whole of Germany has been reduced 50 per cent. since serum treatment was introduced.

The skin rashes, joint pains and other symptoms which occasionally follow the administration of serum have been discussed under "Serum Disease" in the chapter on Anaphylaxis. It is to be noted that fresh serum is more toxic than serum which has been kept for a time; no serum should be used for therapeutic purposes which is less than two months old. By a certain procedure of intermittent heating, the toxicity may also be diminished. These points are carefully attended to in the preparation of the Pasteur Institute serum, and it is perhaps on this account that serious disturbances very rarely follow its administration. It has been observed that symptoms in this country are now much more seldom seen than they were a few years ago—probably owing to more careful methods of preparing the serum.

II. ANTITETANIC SERUM.

The tetanus toxin is found in the filtrate of bouillon cultures of the Tetanus bacillus. It is a most marked nerve poison, acting on the central nervous system. Wassermann has shown that the Tetanus toxin fixes itself to certain tissues even "in vitro." If one mixes an emulsion of brain substance in a test tube with a lethal dose of Tetanus toxin and injects the mixture into a mouse, it remains unhurt. The toxin has become fixed to the brain substance, and is therefore not free to unite in the nervous system of the mouse.

V. Behring found that by immunising animals with Tetanus toxin, he could produce antitoxin.

Clinical Employment. As to the value of Tetanus antitoxic serum, the most widely divergent views are held. Some authorities hold that the correct treatment is to give antitetanic serum in every case of a suspected wound. Others (the minority) hold that the onset of Tetanus and the mortality are uninfluenced by such inoculations.

For the treatment of Tetanus which has already manifested itself, the antitoxic serum, although it has by no means fulfilled the great hopes originally entertained for it, has certainly shown itself of some value. Taking all the cases published up till 1902, the mortality amongst the untreated cases is shown to be 70 per cent., whereas amongst the treated it is 44 per cent.[1] The results are very poor compared with those given by antidiphtheritic treatment, but this is not to be wondered at if one considers how stealthily the disease develops in the depths of the wound, without giving any manifestation till the patient's system may be thoroughly saturated with

[1] These statistics, however, have been questioned.

the toxin. In Tetanus the prognosis is so bad that one must neglect no therapeutic agent, however uncertain. It is well, therefore, to inject in all cases 100-200 units, subcutaneously, in the neighbourhood of the lesion, and then lay bare the nerves of the area and inject as much as possible into them.

III. Botulismus Antitoxin.

The Bacillus Botulinus is the cause of a very deadly form of meat poisoning. The symptoms are due to the absorption from the intestine of the toxins formed by this bacillus.

By repeated injections it is possible to immunise animals against this toxin and then to demonstrate the presence of antitoxin in their serum.

No sufficient statistics of the treatment of human beings with such antitoxic serum are so far forthcoming. However, in such a deadly intoxication the treatment certainly merits a trial.

IV. The Dysentery Antitoxin.

The Dysentery Bacilli of the Shiga-Kruse type produce a true toxin.[1] Dysentery should, therefore, probably be regarded as an intoxication, analogous to the intoxication of Diphtheria. The intestine, where the bacilli have their site, corresponds to the throat in Diphtheria; here the bacteria develop and produce their toxins, and these toxins, when absorbed into the circulation, produce the symptoms of the disease. By injection of the Dysentery toxin into animals an antitoxin can be produced.

[1] For the Flexner type no toxin has yet been demonstrated.

Employment of the Serum in Dysentery in Man. Obviously this antitoxic serum can only be employed for the Shiga-Kruse type of Dysentery. The serum injection must be given as early as possible. Unfortunately the dosage for the various sera on the market has no uniform standard.

Kraus and Doerr make a serum which is supplied in capsules of 20 c.c.m. This represents a single dose. One dose is generally sufficient. In severe cases it is well to double the dose.

It is injected subcutaneously into the flank.

The effects are eminently satisfactory; it should be employed in all cases of this form of Dysentery. For prophylaxis also it seems most efficacious; injections are recommended for persons in circumstances which expose them to the infection.

In cases where a rapidly spreading epidemic is imminent, as in time of war, this serum may prove of the greatest value.

Amongst the antitoxic sera must be mentioned two others which differ from the foregoing, in that the toxins against which they are produced are not secretion products of bacteria, but are the products of higher plants and animals. These sera are Snake Venom Antitoxin and Hay Fever Antitoxin.

V. Snake Venom Antitoxic Serum.

It is possible to immunise animals against snake (cobra and viper) venom by giving them repeated injections of small doses of the venom. The serum of these immunised animals contains antitoxic substance capable of neutralising the venom, and with these antitoxic substances the immunity can be transferred to other animals.

Usually only 10-20 c.c.m. of antiserum require to be injected to cure a person suffering from snake bite, provided the injection be given sufficiently early.

VI. Hay Fever Antitoxin.

According to Dunbar, the cause of Hay Fever is Pollen toxin. This toxin can be extracted from the Pollen ; it is then used for immunising horses, and the antitoxic serum obtained is recommended for the treatment of Hay Fever in man.

The serum is sold in liquid form or in a powder, mixed with milk sugar. It is for local application only.

Under the name of " Pollantin " it is manufactured by Schimmel & Coy., in Miltitz (Leipzig).

The powder is the most convenient form for general use ; it is blown into the nostrils and a few grains are put on the eyelids.

Ill results are sometimes due to excess of " Pollantin " being used.

The statistics seem to show that the " Pollantin " has some value ; about 60 per cent. of the cases are reported to be cured and a certain further percentage distinctly benefited. Many cases, however, seem to receive no advantage from the treatment or are even rendered more susceptible to the attacks.

So much for the purely antitoxic sera. It is to these that serum therapy owes its chief importance. When we come to the *antibacterial sera* the problem of immunisation is much more involved, and the results of the serum therapy are far from satisfactory.

As has been pointed out, only a very limited number of bacteria exert their malign effects through the medium of a secreted toxin. The great majority of the pathogenic bacteria with which we are called upon to fight, *e.g.* staphylococci, streptococci, pneumococci, bac. typhosus, bac. tuberculosis, etc., seem to produce no appreciable exotoxin. Their weapons of attack are quite different, and consequently the weapons of defence used against them must be something other than antitoxins. The marvellous adaptability of the animal body is shown in the extraordinary variety of defensive measures which it can produce.

Our knowledge of these measures is still very primitive.

The antibodies which have so far been demonstrated in antibacterial sera are described in Chapter II.

Besides the antitoxins, there are agglutinins, precipitins, bacteriolysins, bacteriotropins and some less defined amboceptors, whose presence is only recognised by their power to fix complement. It must also be remembered that in addition to these purely humoral defensive arrangements, there is the phagocytic defence—leucocytosis, increased resistance of the leucocytes, and increased phagocytic power (aided by opsonins and bacteriotropins).

When all this is borne in mind, it is easy to realise how complex must be the problem of passively immunising against a bacterial infection.

It is still unknown what part each of these antibodies may play in defending the organism against bacteria. It is therefore impossible to know which substance it is desirable to provide most abundantly in a therapeutic serum, and equally impossible to know how to arrive at a correct dosage or how to estimate the prophylactic or therapeutic power of a serum.

In chronic infections there is the further difficulty that one no longer knows the character of the enemy that one is called upon to fight; the bacteria, through long sojourn in the body, may have acquired powers of resistance to the antibodies, which render them practically a new race.

In testing the effect of the sera upon animals there is the additional complication that the bacteria in question may be very virulent for certain of the laboratory animals and not at all virulent for man, and *vice versa*. For example, a streptococcus which has caused a fatal septicaemia in man may be quite harmless to the laboratory animals, and, on the other hand, a streptococcus which is highly virulent for rabbits or rats may cause no ill effects in the human subject. It is therefore impossible to draw satisfactory deductions of the curative or preventive effect of an antibacterial serum on man, from the results obtained with the same serum in laboratory experiments on the lower animals.

When these facts are borne in mind, it is hardly surprising that passive immunisation with antibacterial sera has made so little headway. That it *has* made headway admits of no denial—the antimeningococcic serum, for example, has conclusively vindicated itself—but that it has achieved no triumphs to compare with that of the purely antitoxic sera, is equally incontestable.

The chief antibacterial sera at present in use for therapeutic purposes will now be briefly dealt with.

I. ANTI-MENINGOCOCCIC SERUM.

A great number of different anti-meningococcic sera are now on the market. The serum of Kolle and Wassermann and that of Flexner are the best known.

Flexner's serum is prepared by the injection of living, fresh cultures of the Weichselbaum Meningococcus into the horse, first subcutaneously and later intravenously.

The sera contain a variety of antibodies—agglutinins, precipitins, bacteriolysins, bacteriotropins and substances which neutralise the endotoxins of the meningococcus (anti-endotoxins).

To estimate the strength of the serum, some manufacturers test the anti-infectious power on animals, others test the bacteriotropic titer, others the toxin-neutralising power and still others the complement-deviating capacity.

Mode of Employment. To employ the serum in meningitis, an intralumbar injection is given, after first withdrawing a corresponding quantity of cerebro-spinal fluid. Subcutaneous and intravenous injections have practically no value, and should never be substituted for the intralumbar method. The direct effect of the serum on the diseased meninges appears to be necessary.

Adults may receive 20-40 c.c.m.

Children may receive 10-20 c.c.m.

It is best to give large doses and to begin as early as possible. It is usually necessary to give repeated injections. Netter recommends systematic re-injection every three or four days, using large doses. Others only re-inject at each exacerbation of the symptoms.

Anaphylactic symptoms are fairly frequent when the serum is used in this way, but no lasting ill-effect has ever been noted.

Results obtained by Flexner in America show reduction of the mortality from 80 per cent. to 20 per cent. by using the serum. The results obtained by using this serum are so good that it must be regarded as imperative to treat cerebro-spinal meningitis by this method.

As a preventive measure, dry pulverised anti-meningococcic serum mixed with milk sugar has been advised by Ruppel for insufflation.

II. ANTI-TYPHOID SERUM.

The serum of Chantemesse is the only serum which has been used on a large scale in the treatment of Typhoid fever in man.

The serum is derived from horses which have been immunised for a long period, partly with soluble Typhoid toxin (whether with endo- or exotoxin is not yet quite clear), and partly with Typhoid bacilli. A very small dose (only a few drops) is used for injection into the patient.

Injection of the serum produces leucocytosis—

in twenty-four hours the white corpuscles may have increased from 5000 to 18,000.

Chantemesse has noted that the serum has a marked effect on the opsonic index of the patient, causing it to rise considerably. The results obtained with this serum are exceedingly encouraging. Among the patients treated by Chantemesse in his hospital the mortality was 4·3 per cent., whilst at the same period the mortality in the other hospitals in Paris, where serum was not used, was 17 per cent. It is important to give the serum early—before the seventh day. The statistics show that no death occurred in patients where the injection was given before the seventh day.

Wright holds that this so-called "serum" of Chantemesse really acts as a vaccine by virtue of the remains of bacilli injected into the horse and still persisting to a certain extent in its serum. He points out that the exceedingly small dose is appropriate for a vaccine and not for a serum, and that the effects—rise in the opsonic index and general improvement of the condition of the patient—are to be reckoned as a triumph of vaccine therapy, not of serum therapy.

III. ANTISTREPTOCOCCIC SERUM.

The value of antistreptococcic serum is still a very debatable question. All the disadvantages which belong to any antimicrobial serum may be quoted against it; and also the further disadvantage that the streptococcus itself is far from being a recognised bacteriological unit. Some authorities believe that all streptococci are of the same family, but the majority now hold the " pluralist " view—that there is a great, possibly an incalculable, number of varieties.

Until bacteriologists have found a method of differentiating the particular streptococci with certainty in a given case, the serum treatment must be haphazard and unscientific.

Numerous sera are prepared: Aronson's, Moser's, Meyer-Ruppel's, Menzer's, etc.

Marmorek, Neufeld and Aronson all believe in the unity of the streptococcus, because they find that a monovalent serum is effective against all streptococci of human disease.

Besredka and others maintain that there are many varieties, and that the serum must be prepared by immunising the horse against as many of them as possible, and thus producing a polyvalent serum. The results obtained with polyvalent sera, however, do not seem to be more reliable than the others.

Doubtless the unsatisfactory history of antistreptococcic serum may be partly accounted for by the absurd uses to which it has been put in the treatment of all sorts of ailments in which the streptococci play a purely hypothetical part. For example, in Puerperal Fever, the antistreptococcic serum is often given when the infection is due to an entirely different bacterium. For streptococcic infections it is much more advisable to use a vaccine prepared from cultures of the patient's own infecting bacterium.

ANTISCARLETINAL SERUM.

This is a variety of antistreptococcic serum prepared by Moser by immunising horses with streptococci, which he cultivates from the heart blood of children dead of Scarlet Fever.

Opinions are greatly divided as to the value of this serum. It appears from the most recent researches that the specificity of the Scarlet Fever Streptococcus is far from being proved. The true causative micro-organism of Scarlet Fever is still to be discovered.

The serum may be useful in Scarlet Fever to combat the effects due to secondary invasions of streptococci.

IV. ANTIPNEUMOCOCCIC SERUM.

A polyvalent antipneumococcic serum has been prepared by Römer and others, and is manufactured by Meister, Lucius, and Brüning (Höchst), and by Merck (Darmstadt). Of its value in Pneumonia nothing definite can be said ; the statistics are quite insufficient. In Ulcus Corneae Serpens, however, it seems to be of considerable value. 200-400 units are injected and the dose is repeated on the second or third day. A few drops of the serum are also instilled into the eye every two hours.

Horder, in America, has tried to prepare a serum which would be of use in cases of pneumococcic endocarditis, but has failed. According to Rosenow " the results of the animal experiments make the outlook for a specific serum therapy very gloomy. The more one learns of the mechanism of the infection in these cases, the more difficult the discovery of a specific therapy would seem to be."

V. ANTITUBERCULOUS SERUM.

The serum of Maragliano has been considerably used in Italy, and good results are reported in early cases. Doses of 1-5 c.c.m. are given subcutaneously every second day for several months.

The serum of Marmorek, however, is the best known of the antituberculous sera. It is prepared by immunising horses with tubercle bacilli, and then with various kinds of streptococci, so that the serum is both antituberculous and polyvalent antistreptococcic. It is given in doses of 10 c.c.m. subcutaneously three times a week, or 20 c.c.m daily per rectum. The latter method is advisable in order to avoid symptoms of "serum disease."

Opinions regarding the value of the serum are still very divergent.

Citron recommends its use in cases of persistently high fever, and also in cases where Tuberculin treatment has been tried without success. In some cases he combines the Serum- and Tuberculin-therapy, commencing with Serum, then giving both together, and finally giving Tuberculin alone.

Sensitised tubercle bacilli (S.B.E.) have been recommended by Meyer—prepared by Meister, Lucius and Brüning.

VI. ANTI-PLAGUE SERUM.

This is prepared by immunising horses by intravenous injections of B. Pestis, using dead and then living cultures. The serum has a preventive action in all laboratory animals susceptible to Plague. In man it has a preventive action which is quite marked, but unfortunately is very transitory, all immunity disappearing after ten days.

The statistics for many corners of the globe would appear to be in favour of the serum ; in India, however, this is not the case ; there, undoubtedly, the serum treatment of Plague has not given anything like the results expected. The extreme susceptibility of the Hindu to Plague has been given as the explanation. It also seems, however, that in the cases where unsatisfactory figures have been reported there may be other explanations of the failure—too late administration, doses too small, and the use of the subcutaneous method of injection instead of the intravenous. Be this as it may, the fact remains that where every kind of case was treated and no special selection made, the mortality was not affected by the serum treatment. Yersin's statistics, which were comparatively encouraging, were based on the treatment of selected cases.

Employment. *Early* injection is insisted on by the advocates of the treatment.

Large and repeated doses are necessary—*e.g.* 100 c.c.m., followed in twenty-four hours by another 100 c.c.m.

The *intravenous* route is much the best.

VII. ANTI-CHOLERA SERUM.

This has not yet been sufficiently put to the test in the treatment of Cholera in man. It has, however, a marked effect in animal experiments and must be given a trial in human therapeutics.

A toxin has been prepared by Kraus from cultures of Cholera vibrios, and animals have been immunised with this and an antitoxin has been produced. Attempts have been made to use this serum in the treatment of Cholera in Russia, and the results reported are encouraging.

VIII. Anti-anthrax Serum.

Anti-anthrax Serum, prepared by the immunisation of horses and asses, has been used both as a preventive and therapeutic agent for oxen, sheep, horses and pigs. It has also been tried in the treatment of Anthrax in man. For this purpose Sclavo's serum is used; 30-40 c.c.m. should be injected subcutaneously—the injections being distributed over three or four different sites. If there is no improvement, the dose should be repeated after twenty-four hours.

In very severe cases it is better to inject intravenously 10 c.c.m. Bandi reports two cases where he gave 150 c.c.m. intravenously, with good results.

Since the introduction of Sclavo's serum, the statistics show that in Italy the mortality has been reduced from 24 per cent to 5·3 per cent.

B. VACCINE THERAPY.

Prophylactic Vaccination.

In 1798 Jenner published the results of his researches on small-pox vaccination. But the true father of vaccines was not an Englishman. The scientific discovery belongs to Pasteur and dates from about 1879—80 years after Jenner's announcement.

The original method of immunising against small-pox was to inoculate the disease from a small-pox patient into a healthy person. This generally caused only a mild attack, and the person was afterwards immune to the disease. The disadvantage, however, was that each inoculated person was a source of infection for others, and a very grave danger to the community. It was Lady Mary Wortley Montagu who, about the year 1720, first introduced this method of small-pox inoculation into England. She had been living in Constantinople where small-pox was rife, and had seen inoculations practised by the Turks, chiefly with a view to preserving the beauty of the young Turkish and Circassian women. She had her own son and daughter inoculated, and when she returned to England she encouraged the practice there. From her influence can be traced the rapid spread of inoculation in this country and in Western Europe.

But, as has been said, this practice was dangerous, and it was not until Jenner discovered his method of vaccination that a satisfactory process of immunisation against small-pox was introduced.

Jenner was a physician practising in Berkeley (Gloucestershire), and he frequently used the method of inoculation against small-pox amongst his patients. He noticed, however, that certain people were insusceptible to such inoculations—when inoculated from a small-pox case they showed no result. Observation then showed that these people who were immune were always dairy or farm folk, accustomed to work with cows ; in fact, were always people who had already had cow-pox. Jenner then thought of inoculating cow-pox instead of small-pox, in order to immunise a person against small-pox. The enormous success of this method is known to all.

These observations of Jenner, however, were too empirical to be classed as the beginnings of a definite science, and the scientific discovery of vaccines must be attributed to Pasteur.

With all his absorption in science Pasteur was always a warm-hearted humanitarian, and the great dream of his life was that his researches might lead to something which would lessen the sufferings of his fellow-men. So, while he was making his bacteriological discoveries, he had all the time a watchful eye for any ray of light in that direction. He knew that one attack—even a mild attack—of many infectious diseases protected the patient for the future against that disease. He also saw that Jenner had found a method of artificially protecting against a severe disease by making the patient suffer from a milder form. Through his laboratory work he came to realise that these infectious diseases are probably all due to bacteria, and the absorbing question then came to be : Is there no method of weakening these bacteria, so that they will give a mild form of the disease, and that a person inoculated with this mild form may become immune to the original virulent form of the disease ? In the end, the discovery came, almost by accident—the discovery of the attenuation of viruses, upon which all the rest hangs.

Before going for his holidays in 1879, Pasteur had been working with the micro-organism of chicken cholera; when he returned after the holiday and examined his cultures, he found that they had become innocuous—that fowls could bear inoculations of what formerly would have been a lethal dose, without any ill effect. Pasteur immediately grasped the far-reaching importance of this. His first thought was that he might here, in this attenuated micro-organism, have a vaccine—that he might, by inoculating this weakened germ, be able to produce a very mild and modified form of chicken cholera, which would afford protection to the fowl against the severe form of the disease.

This proved to be the case; hens which had received an injection of the attenuated bacteria recovered after a slight illness, and, if subsequently inoculated with fully virulent germs, they survived without injury.

These two discoveries, then—the possibility of attenuating the virulence of a virus, and the possibility of conferring immunity to a disease by the inoculation of the attenuated virus—made the foundation for all the vaccine work of the future.

Pasteur's next endeavour was to find a vaccine for Anthrax—that terrible disease which was causing enormous losses to cattle owners in various parts of the world. The difficulty here was that the bacillus formed spores, and these spores maintained the virulence of the original bacteria. By persevering experiment, however, he discovered a means of suppressing the spore formation; by growing B. Anthracis at a temperature of 42° C., he obtained cultures free from spores, and the bacteria themselves became attenuated after a certain time. With these attenuated bacteria he found that he could effectively vaccinate sheep and cattle, and so preserve them against an attack of the disease.

One of the most dramatic stories in the history of science is the account of how Pasteur demonstrated this to the public. Some rather venomous critics, anxious to humiliate Pasteur, and having heard of his ability to prevent Anthrax in laboratory experiments, sent him a public challenge to come and carry out the experiment on a practical scale at a farm in the country. Some farmers offered to put sixty sheep at his disposal. The challenge was immediately accepted, and Pasteur drew out a plan of exactly what would happen, safeguarding himself behind no half-statements, but boldly promising complete success: twenty-five sheep were to be vaccinated, twenty-five should remain unvaccinated; a fortnight later all the fifty were to receive a lethal dose of the fully virulent Anthrax; the twenty-five non-vaccinated would die; the twenty-five vaccinated would remain alive and well. The remaining ten sheep were to serve as controls.

The challenge and its acceptance were widely advertised in the journals, and much joking was made at Pasteur's expense. Excitement ran high, and a huge crowd, including doctors, veterinary surgeons, journalists, farmers, etc., accompanied Pasteur to the farm (Pouilly le Fort) where he was to make the final test by inoculating the deadly Anthrax. Next day a telegram announced to Pasteur that one of the vaccinated sheep had a temperature, and then, for the first time, his courage flagged. He spent a sleepless night, walking about in great agitation. Next day he went to the farm, again followed by the crowd, and found all the vaccinated animals well! Of the unvaccinated, twenty-two were dead, and the others died during the following night. Pasteur's triumph was complete, and the possibility of preventive vaccination was demonstrated to the world.

The next success was with Hydrophobia. Here the difficulties were still greater, for although it was known that the virus existed chiefly in the central nervous system of the dog suffering from rabies, and that inoculation of this nerve tissue could always produce the disease in other animals, no definite micro-organism could be isolated.

New methods of attenuation had to be sought for this virus.

Pasteur first succeeded in finding a method of greatly augmenting the virulence—by successive inoculations from one rabbit to another. The spinal cords of these rabbits contained the virus in various degrees of virulence. He then found that the virulence could be lessened by drying the cords over caustic potash; the longer the cords were exposed, the less the virulence, till a point was reached (after fourteen days' drying) at which no virulence remained. Being now in possession of the virus in all grades of increased and attenuated virulence, Pasteur commenced the experimental vaccination of animals. By using emulsions of these cords, commencing with the least virulent and each day going on to a more virulent, he was able to render dogs absolutely immune to rabies. He hesitated very much to apply this experiment to human beings, but his hand was forced when a child was brought to him, who had been severely bitten by a mad dog two days before. He dared not hold back; he was bound to give the child its one chance of cure. The result was absolutely successful, and from that arose the anti-rabic vaccination service in Paris, and later the Pasteur Institute in Paris, and similar Pasteur Institutes over the whole world (except England).

The possibility of immunising against the disease in man after he has been bitten, is due to the fact that the incubation period of Hydrophobia is very long—generally not less than six weeks, and sometimes even as long as 584 days.

The treatment usually lasts fifteen days. On the first day the patient receives an emulsion of the spinal cord of a rabbit dried over caustic potash for fourteen days, next day an emulsion of cord dried for thirteen days, and so on, the virulence of the injected material being greater each succeeding day. Finally fully virulent cord is injected.

When the bites are about the head and face, even more vigorous treatment is given, as the danger is greater.

Besides these mentioned, Pasteur's vaccines against Swine Erysipelas, Symptomatic Anthrax and Rinderpest are well known.

This, then, is the magnificent foundation for vaccine treatment laid by Pasteur.

It is to be noted that, with true courtesy, Pasteur adhered to Jenner's nomenclature—he wished to give Jenner the honour of having originated this idea.

The word vaccine is from the Latin *vacca, vaccinus* (a cow); cow-pox was called "vaccinia," the cow disease, and Jenner called his protective inoculation "vaccination."

Pasteur then extended the use of the word vaccine to include any attenuated virus inoculated to produce protection against the fully virulent virus.

Before going on to its later developments, it will be well to explain that artificial immunisation may be carried out in four different ways:

1. By inoculating living micro-organisms.
2. ,, ,, dead ,,
3. ,, ,, bacterial extracts.
4. ,, ,, bacterial toxins.

The first method gives rise to an immunity which is very pronounced and of long duration. The disadvantage, however, is obvious—it is the difficulty of regulating the virulence and the dosage of the employed bacteria, so that a sufficient immunisation response may result, and, at the same time, all risk of setting up a general infection through the multiplication of the injected bacteria, may be avoided. To this end, the micro-organisms must be attenuated in their virulence. How effectively this was done by Pasteur has just been seen.

Since the discovery of the anti-rabic vaccination in 1885, there have been endless endeavours to prevent and treat other infective diseases according to the same principle. Preventive inoculations have been carried out on a large scale chiefly against cholera, typhoid fever and plague.

TYPHOID FEVER.

For prophylaxis against Typhoid Fever, inoculations have been widely employed.

Wright and Pfeiffer, working independently at the same time, both used dead cultures.

The method of Wright has been largely used in the British Army, for a time with doubtful success; but according to the most recent figures the success is absolutely convincing in India, America, and Japan.

Forty-eight-hour old bouillon cultures of B. Typhosus are used and are killed by heating for an hour at 53° C. 0·5 per cent. Carbolic Acid is then added as a preservative. Usually two inoculations are given with an interval of ten days. For the first inoculation the dose is 500 millions, for the second, 1000 millions.

During the South African War, when this method was given a fair trial, the results were not so good as had been expected. Leishman, however, who now has charge of the preparation of these army vaccines at Netley, has discovered some of the reasons for failure, and has been able to obtain much more favourable statistics. He points out, for example, that some of the vaccine used during the Boer war was too old, some had been injured by sterilising at too high a temperature (60° instead of 53° C.), and some by having Lysol added to it while it was still hot.

After the injection of the vaccine, there is, according to Wright, a "negative phase," preceding the appearance of bactericidins, agglutinins, etc., in the blood; this lasts for about seven days. Every person inoculated ought to be protected from infection during this time. The method of preventive vaccination is therefore not applicable in times of epidemics. Leishman, on the other hand, finds no "negative phase" succeeding the inoculation. According to Wright, the immunity conferred lasts about three years.

The statistics of the Germans during the war in German South-West Africa (where the vaccine of Pfeiffer and Kolle was used), are quite similar to those of the British Army during the Boer War.

The summing-up of Leishman in his report on Anti-typhoid inoculations may be quoted as giving a very temperate view of the present position of the subject. He says, "I am very far from suggesting that the number of cases so far reported establishes the value of vaccine therapy in Enteric Fever, but I submit that the results obtained are good enough to encourage a wider trial of the system, which I believe to have a sound scientific foundation."

Some work recently published by Metchnikoff and Besredka throws an important light on this subject, and seems to offer a hope of a much more reliable method of vaccination against Typhoid Fever.

Working with anthropoid apes—the only laboratory animals which can be made to contract Typhoid Fever—they have shown that it is impossible to vaccinate them against this fever by means of dead bacilli. On the other hand, the subcutaneous injection of living Typhoid bacilli confers on the chimpanzee an assured immunity. These injections of living Typhoid bacilli under the skin, however, give rise to an intense local and general reaction, but this disadvantage can be annulled by a preliminary sensitisation of the bacilli (leaving them in contact for some time with an anti-typhoid serum, then centrifugalising and washing in saline). These sensitised bacteria when injected cause a very slight local reaction and practically no general reaction, but nevertheless confer immunity on the chimpanzee.

So far, Metchnikoff and Besredka report only two cases of such vaccinations on human beings, but in these cases the vaccine was perfectly harmless.

It is quite possible that this vaccination with living virus may yet find a wide application in the prevention of Typhoid fever in man.

PLAGUE.

Preventive inoculation against Plague has been carried out on a large scale in India, by Haffkine. Killed bouillon cultures of B. Pestis were employed; these cultures had grown for a month. Two inoculations were given.

The method has undoubtedly some value—it considerably reduces the liability to Plague and, even among those who are unfortunate enough to take the disease, in spite of having been inoculated, it greatly modifies its course and reduces the mortality.

Doctors, nurses and others going into contact with Plague should be inoculated.

The German Plague Commission strongly recommends the use of twenty-four hour old agar cultures instead of these very old bouillon cultures.

Kolle and Strong have also employed living cultures of greatly reduced virulence for the immunisation of man; no ill effects of the inoculation are reported. The statistics are still inadequate.

THERAPEUTIC VACCINATION.

Of recent years, active immunisation by means of vaccines has become much more widely employed, thanks to the brilliant work of Sir Almroth Wright. He has demonstrated that a vaccine may be used not only as a prophylactic measure, but also as a therapeutic agent in already existing disease. According to Wright's view, "the fundamental principle of vaccine therapy is to exploit in the interest of the infected tissues, the unexercised immunising capacities of the uninfected tissues."

He believes that antibacterial substances are elaborated at the site of inoculation of the vaccine, that these are produced in excess and that they pass into the blood and are conveyed to the seat of infection.

Experiments of Hektoen and others go to show that this is a fallacy—antibacterial substances are not produced to any appreciable degree at the site of inoculation—but this does not affect the question of the practical value of Wright's vaccines.

Opinions as to their value as therapeutic agents unfortunately have to be founded on individual clinical impressions, and not on any scientific data. Thus, even to-day, when the vaccines have had a period of probation in England of about seven years, the extent of their usefulness is still an open and debatable question. Wright's original method of controlling each inoculation by an estimation of the opsonic index raised hopes of scientific accuracy which have not been fulfilled.

In the section on Opsonins (page 55), it has been pointed out that there are many reasons which render it impossible to take the patient's opsonic index as a guide in vaccine treatment. It has now been largely laid aside, even by the vaccine therapist, and clinical indications and empirical rules of dosage must take its place. When all this is said, however, the significant fact remains that the medical men who have had the largest experience of vaccine therapy are most strongly convinced of its value.

In chronic staphylococcic infections, such as furunculosis, sycosis and acne, there is great unanimity regarding the efficacy of staphylococcic vaccines. In cystitis, due to B. Coli, the results obtained seem also to be almost uniformly good.

In a great variety of localised infections where the causal organism can be isolated and a vaccine prepared from the cultures, there is overwhelming evidence of successful results. In severe generalised infections, such as malignant endocarditis, pyaemia, puerperal septicaemia and pneumonia, it is, of course, more difficult to get convincingly good statistics. This may be largely due to the fact that the vaccine is frequently not employed until everything else has failed and the patient is moribund. But almost every worker who has given vaccines a fair trial in these conditions has been convinced of their beneficial effect, and has seen lives saved which were otherwise despaired of.

For further information on this subject, the student must refer to clinical text-books.

The method of preparing a Wright's vaccine is as follows: The causal organism is isolated from the pus, urine, sputum, etc., and slope cultures of the pure germ are made on a suitable solid medium—ordinary agar, serum agar, etc. After twenty-four hours incubation at 37° C., these cultures are emulsified in normal saline solution (·85 per cent.). A few drops of the saline are allowed to moisten the growth, which is then rubbed down by means of a glass capillary tube sealed at the end. More saline is then added till a thick opalescent emulsion is obtained, which must be as uniform and as free from clumps as possible. This is then poured off into a sterile glass centrifuge tube containing some sterile glass beads, and is put in a shaking machine to shake for several hours. The emulsion is then centrifugalised for a few minutes to drive down any remaining clumps and is ready to standardise and sterilise. It is best to remove a small quantity into a separate tube to be afterwards used for the standardisation, as the process of sterilising may interfere with the staining properties of the bacteria, and thus affect the count in the standardisation.

The emulsion is sterilised by placing in a water-bath for an hour at a temperature which varies with the bacteria in question. For staphylococci, 1 hour at 65° C. is necessary. For Bac. Typhosus, Leishman recommends a temperature of only 53° C., but this will not prove sufficient to kill all strains of Bac. Typhosus. The sterility of the vaccine is tested, after it has cooled, by allowing a few drops to run over the surface of an agar slope (serum agar in the case of streptococci and pneumococci, and 0·6 per cent. acid ascitic agar for gonococci), and setting the slope in the incubator at 37° C. for twenty-four hours. If any colonies are found, the vaccine must be rejected and a fresh one made.

The standardisation is carried out by the blood count method devised by Wright. In this method, some of the bacterial emulsion is mixed with an equal quantity of human blood and the number of bacteria to be counted microscopically in a given space is compared with the number of red blood corpuscles in the same space. Advantage is here taken of the fact that the number of red corpuscles in adult human blood is fairly constant—5 million per c.m.m.—and that a calculation in simple proportion will thus give the number of bacteria. A capillary pipette, fitted with a rubber teat, is marked with a blue pencil at a point about one inch from the end. Equal volumes of blood from the finger, of bacterial emulsion and of sterile citrated saline solution (1·5 per cent. sodium citrate in ·85 per cent. sodium chloride) are sucked into the pipette, separated by air bubbles from each other, and are then blown out on a clean glass slide and thoroughly mixed by sucking into and blowing out of the pipette. A drop of the mixture is then transferred to one end of a clean glass microscope slide, and another slide is used to spread it out into a thin smear. This is allowed to dry and is then stained by Leishman's stain, or is fixed in saturated solution of mercuric chloride and stained by carbol-thionin. The estimation is then arrived at by counting the number of red corpuscles and of bacteria respectively, contained in a succession of 20-40 microscopic fields. The enumeration is much facilitated by the use of an Ehrlich eye-piece, which cuts off the field into a square of any desired size. Failing this, a small square may be cut out of a piece of paper and this dropped into the ordinary eye-piece. The calculation is: $\frac{\text{number of bacteria counted}}{\text{number of red corpuscles counted}} \times 5000$ = number of millions of bacteria in 1 c.c.m. of the vaccine.

Carbolic Acid (0·5 per cent.) is now added as a preservative and the vaccine is tubed in small glass bulbs each containing 1 c.c.m., which are then sealed in the blow-pipe and scored across the neck with a glass knife to facilitate opening when required.

C. THERAPEUTIC EMPLOYMENT OF TUBERCULIN.

The therapeutic employment of tuberculin must be briefly considered under "vaccine therapy."

The many accidents following the use of Koch's old tuberculin in its early days caused it to fall into disrepute for a considerable time. Of recent years, however, the subject has been better understood, and some of the reasons for failure have been discovered; newer and probably safer forms of tuberculin have also been introduced, and the tuberculin treatment of almost all forms of tuberculosis has now a wide vogue. For the treatment of tuberculous glands, lupus, etc., tuberculin has been widely used; it is now also employed to a great extent in the treatment of phthisis in sanatoria and elsewhere.

A great variety of preparations from the tubercle bacillus have been offered for therapeutic purposes. Only the most important will be mentioned here.

I. OLD TUBERCULIN.

The mode of preparation and the constitution of this are discussed under tuberculin-diagnostic (page 148). It is the filtrate of a 4-5 weeks old culture of tubercle bacilli in glycerinated bouillon, which is sterilised by steam and reduced to one-tenth of its volume. This form of tuberculin is still largely used for therapeutic purposes on the Continent, but in Britain its employment is very limited.

II. NEW TUBERCULIN (T.R.).

This was introduced by Koch in his efforts to make a preparation free from the undesirable properties of old tuberculin. Tubercle bacilli are thoroughly triturated and then treated with water to dissolve out all soluble products. The resulting solution is T.O. From the insoluble residue T.R. is prepared by alternately treating with water and centrifugalising. By repeating this process several times, a number of very fine emulsions are obtained, and these are finally mixed together to form T.R.

Tuberculin T.R. thus contains the part of the tubercle bacilli which is insoluble in water.

This form of tuberculin is now largely employed as a therapeutic vaccine. It is well borne by patients; if appropriate doses are employed, fever and symptoms of a general reaction are very slight or altogether absent.

The preparation may be made either from the bovine or the human type of tubercle bacillus.

III. New Tuberculin Bacilli Emulsion (B.E.).

This contains both the soluble and insoluble components of the tubercle bacilli. The living bacilli are ground down into a fine powder, and this is then treated with saline solution. The insoluble part is left to settle (not centrifugalised). 50 per cent. glycerine is added as a preservative.

This preparation also is now widely used for therapeutic purposes. It may be prepared either with the human or bovine type of bacillus.

IV. Tuberculosis Sero-Vaccine (S.B.E.).

This is an emulsion prepared from sensitised tubercle bacilli, *i.e.* tubercle bacilli which have been placed in a quantity of serum from an animal immunised against tuberculosis. The bacilli are centrifugalised free from the serum, repeatedly washed with saline solution and finally emulsified in 50 per cent. glycerine water.

The principle of this method of treatment with sensitised bacteria was first suggested by Besredka in connection with typhoid bacilli.

V. THE TUBERCULIN OF BERANECK.

This is a mixture of two tuberculins, the one a filtrate of bouillon cultures of tubercle bacilli, and the other an extract of tubercle bacilli with 1 per cent. phosphoric acid.

VI. THE TUBERCULIN OF DENYS ("bouillon filtré").

This is a filtrate of bouillon cultures of tubercle bacilli. It differs from old tuberculin in not being subjected to heat and not being reduced to one-tenth of the original volume. It is identical with the T.O.A. (original old tuberculin) of Koch. The latter worker, however, never recommended its use as a therapeutic agent.

VII. PERLSUCHT TUBERCULIN ORIGINAL (P.T.O.).

This is of the same constitution as VI., but is prepared from bacilli of the bovine type.

VIII. ALBUMOSE-FREE TUBERCULIN.

Tubercle bacilli are grown in an inorganic medium, containing no peptone. The object is to have no albumoses in the final product. With this preparation larger doses may be given without producing a severe reaction.

CHAPTER VI.

IMMUNITY REACTIONS EMPLOYED FOR DIAGNOSTIC PURPOSES.

A. THE AGGLUTININ TEST.

THE value of the agglutination test in the diagnosis of disease is limited chiefly to Typhoid and Paratyphoid Fevers, and, in a less degree, to Cerebro-Spinal Meningitis and bacillary Dysentery.

It is especially useful in atypical cases of Typhoid Fever where, clinically, the diagnosis cannot be made with certainty. It is also valuable for the diagnosis of " Typhoid Carriers."

As the question of the Agglutinins is fully discussed in Chap. II., it only remains to describe the technique of the agglutination experiments.

The microscopic method is generally employed for the Gruber-Widal reaction for Typhoid Fever, as the quantity of blood available is frequently very small. Where a sufficient amount of blood is supplied, the macroscopic method may be used, and this is the method to be generally preferred for scientific research.

I. THE MACROSCOPIC METHOD.

For this, one requires (*a*) a homogeneous emulsion of the bacteria ; and (*b*) the serum to be tested.

To produce a homogeneous emulsion, *i.e.* one in which the bacteria are uniformly suspended, and where there are no clumps, is the only really delicate part of the experiment. Bouillon cultures may be employed, or emulsions of growths on agar slopes. If the bacteria grow in bouillon forming a uniform cloudiness—as with B. Typhosus, B. Coli, B. Paratyphosus, etc.—the bouillon cultures may be used. The bouillon cultures must not be more than eighteen to twenty-four hours old. If agar cultures are used, the emulsion must not be too thick. Killed cultures are frequently used—killed by heat, formaline, toluol, 5 per cent. carbolic acid, etc. Of these dead cultures, that which bears the name of the " Ficker Diagnosticum " is most to be recommended ; the results given by it are thoroughly satisfactory.

The " Ficker Diagnosticum " (Merck, Darmstadt) is a preparation containing the dead bacilli ; the exact mode of its production is not made known. It will be found extremely useful for the physician who wishes to carry out his own " Widal " investigations, as it is perfectly safe to work with, and enables one to dispense with the trouble of preparing cultures and emulsions.

To collect the blood, a convenient method is to use a Wright's capsule, as employed in opsonic estimations. The arm is hung down or swung, to cause congestion, a finger is bandaged from the root to near the nail, and a few sharp pricks are made with the point of the glass capsule into the back of the finger at the root of the nail. The short end of the capsule is applied to the drops of blood, and when sufficient has flowed in to half-fill the capsule, the other end (distal from the blood) is heated for a second to expel air and the point sealed in the flame. As soon as the capsule cools, the blood flows around the bend of the tube away from the end at which it entered and this end is then also sealed in the flame.

For the macroscopic method, where a larger quantity of blood is required, it is best to draw it off with a syringe from a vein in the bend of the elbow. An all-glass syringe is to be preferred.

When the blood is collected, it is set aside to coagulate, then centrifugalised. The Wright's capsule is very conveniently hung by its arm in the bucket of the centrifuge. The serum is then pipetted off with a capillary pipette.

Dilutions of the serum are made with normal saline solution (·85 per cent. sodium chloride), or with bouillon ; dilutions of 1 : 10, 1 : 50, 1 : 100 are usually sufficient for the clinical diagnosis of Typhoid. A series of four small tubes is set in a rack ; into each is put ·5 c.c.m. of the bacillary emulsion. Then into No. 1 is put ·5 c.c.m of normal saline solution, into No. 2, ·5 c.c.m. of the 1 : 10 dilution, into No. 3, ·5 c.c.m. of the 1 : 50 dilution, and into No. 4, ·5 c.c.m. of the 1 : 100 dilution. Thus :

Bac. Suspension.	Serum Dilution.	Normal Saline Solution.
1. 0·5 c.c.m.	....	0·5 c.c.m.
2. ,, ,,	0·5 c.c.m. of 1 : 10 dilution	....
3. ,, ,,	0·5 c.c.m. of 1 : 50 dilution	....
4. ,, ,,	0·5 c.c.m, of 1 : 100 dilution	....

It is well to do also a control series with normal serum.

Place the tubes for two hours in the incubator at 37° C. Then leave at room temperature for six hours before reading off the result.

Tube No. 1 is the control and must show no agglutination. For the diagnosis of Typhoid, the result is generally considered positive if agglutination is given by the dilution of 1 : 100; if given only up to 1 : 50, a probability of Typhoid may be assumed, but one is hardly justified in giving a positive diagnosis.

II. THE MICROSCOPIC METHOD.

Having obtained the serum as described above, make the dilutions (1 : 30, 1 : 50, 1 : 100) with normal saline solution or with bouillon. Take a platinum loopful of the serum dilution and a similar platinum loopful of the bacterial emulsion, and mix together on a slide. Then put a drop of the mixture on a clean cover glass and invert it over a hollow slide, the hollow having been previously surrounded with vaseline. This "hanging drop" preparation is now observed microscopically, first with the low power and then with the oil immersion.

Read off the result after $1\frac{1}{2}$-2 hours at room temperature. The reaction is "positive" when large clumps of agglutinated bacilli are seen and the few isolated bacilli are immobile.

A control preparation without serum must always be made.

PFAUNDLER'S THREAD REACTION.

This may sometimes be used instead of the ordinary agglutination test.

Pfaundler noted that when a bacterium is grown in the corresponding immune serum, it may develop in a peculiar manner—growing into long filaments or "threads." This was found to be the case with B. Typh., B. Coli, Vib. Cholerae, etc.

Mandelbaum advises the following technique: 2 g. sodium citrate are dissolved in 100 c.c.m. bouillon. This is divided up into test-tubes—about 5 c.c.m. to each—and these are sterilised.

For the experiment a tube is then infected with a loopful of a bouillon culture of B. Typh. A drop of the patient's blood is drawn into a capillary pipette and about ten times the quantity of the infected citrated bouillon is also drawn in. The fluid is sucked up in the tube and the end is sealed in the flame. The preparation is thoroughly shaken and then kept in the incubator at 37° C. for forty-eight hours. Then the corpuscles will be found to have sunk to the bottom and left a clear fluid above; this fluid is examined microscopically in "hanging drops." If the serum is from a Typhoid patient, the bacilli will be found quite immobile, and in the form of long filaments. If the case is not Typhoid, the bacilli retain their normal appearance and motility.

B. THE PRECIPITIN TEST.

The precipitin test has not so far attained any great significance in clinical diagnosis, and so the technique will not be described here. For the diagnosis of bacteria, its interest is scientific rather than practical, as the agglutinin test covers almost entirely the same ground and is much more easily carried out.

Maragliano's precipitin test for the diagnosis of malignant tumours, and the tests of *Kelling* and others must await confirmation before they can be of practical interest to the clinician.

Vincent and Bellot advise the " precipito-reaction " in the lumbar fluid as being of considerable value in the diagnosis of Cerebro-Spinal Meningitis. It is simple and demands no elaborate technique.

The cerebro-spinal fluid is centrifugalised till it is quite clear; then two tubes are taken and into one is put 50 drops, into the other 100 drops of this clear fluid. One drop of Meningococcic serum is added to each tube. (The Wassermann Meningococcic serum is the best for this test; it may be obtained from the Kgl. Institut für Infektionskrankheiten in Berlin.)

The tubes are kept for 8-12 hours at a temperature of 50-53° C.

In cases of Cerebro-Spinal Meningitis a precipitate forms. In the case of normal serum, or serum from other forms of Meningitis, no cloudiness results. This reaction is obtainable within the first twenty-four hours of the illness and persists till the twelfth, or even as late as the twentieth day.

C. OPSONIC ESTIMATIONS.

The question of the clinical value of opsonic estimations has already been discussed (page 55).

Here it is only necessary to describe the technique, and to emphasise the point that, whatever value such tests may have in the hands of the experienced worker, they are absolutely worthless when performed by the unskilled. The sources of fallacy are so numerous, that here, perhaps more than in any other sero-diagnostic test, a patient and well-trained worker is a *sine qua non*.

For the Wright Opsonic Index Experiment the following are necessary :

1. A bacterial emulsion which is perfectly homogeneous, contains no clumps, and is not too thick,
2. The patient's serum and several normal sera, to serve for the control,
3. A suspension of washed, human leucocytes in normal saline solution.

1. THE BACTERIAL EMULSION.

For most micro-organisms, young agar cultures (not more than twenty-four hours old) are employed. The emulsion is made with normal saline solution. Into a tube, containing several c.c.m. of normal saline, is introduced a platinum loopful of bacteria from the surface of an agar culture. The platinum loop is not allowed to enter the solution, but is brought against the dry inner side of the tube, which is then tilted till the fluid just touches the loop. The loopful of bacteria is now rubbed against the tube wall, which is merely moistened by the slightest contact of the fluid. In this way a homogeneous emulsion is made which is disseminated through the bulk of the solution by shaking.

For preparing an emulsion of tubercle bacilli, Wright recommends the use of 1·5 per cent. salt solution instead of normal saline, and bacilli which are the residue obtained from the preparation of old Tuberculin (obtainable from Meister, Lucius and Brüning).

2. The Serum.

This is best collected in a Wright's glass capsule. The method has already been explained under the "Agglutination Test" (page 115). After leaving the blood to clot, the capsule is hung by its arm in the bucket of the centrifuge and centrifugalised till the serum is clear.

3. The Leucocytes.

These are generally obtained by the experimenter from his own blood. The back of a finger, near the nail, is pricked several times with the glass point of a Wright's capsule, newly drawn out in the flame to make it quite sharp and also sterile.

A number of drops of blood (varying with the amount of leucocytes required for the tests in hand) are collected in a centrifuge tube, which is two-thirds full of a solution of Sodium citrate (1·5 per cent.) in normal saline (·85 per cent. NaCl).

To get the blood to flow easily the arm should be swung round several times in a circular fashion from the shoulder, and the finger should be bandaged from the root to near the nail.

The blood is shaken up with the citrated salt solution (the object of the sodium citrate is to prevent coagulation) and is then centrifugalised till all the corpuscles lie at the bottom. The fluid is then carefully removed with a glass syphon. The tube is filled up to the former level with normal saline solution, the corpuscles are shaken thoroughly through it, and the mixture is once more centrifugalised. The clear fluid is again syphoned off the corpuscles, fresh saline solution is added and the centrifugalisation repeated once more. After two or three washings in saline, the corpuscles are ready for use. The saline solution is all carefully syphoned off and the corpuscles are mixed by sucking up and down with a pipette. (Formerly it was advised to skim the white corpuscles from the surface of the red, but it is now recommended to mix them as above.)

The experiment is now performed as follows: A capillary pipette, fitted with a good rubber teat, is marked with a blue pencil at a point about 1″ or 1½″ from the distal end. This mark permits the measuring of equal volumes of the bacterial emulsion, serum and leucocytes. By means of the rubber teat, one draws up leucocytes to the level of the pencil mark; an air bubble is then admitted to separate the leucocytes from the bacterial emulsion, which is now drawn in as far as the pencil mark; another air bubble follows and finally the serum is drawn in as far as the mark. These equal quantities of the three ingredients are then blown out on a clean glass slide, and thoroughly mixed by drawing in and blowing out of the pipette a good many times. The mixture is then sucked up into the pipette right away from the end, which is finally sealed in the flame. The pipette is then kept in the incubator at 37° C. for 15 minutes. Rosenow recommends that during this time the preparation be constantly shaken by means of a small shaking machine which can be put in the incubator. At the end of this time the pipette is taken out, the end broken off, and the mixture blown out on a slide and again thoroughly mixed by sucking in and out of the pipette.

Film preparations are made by putting a small drop of the mixture at one end of a perfectly clean glass slide and spreading with the edge of another slide held over the drop at an acute angle, and slowly drawn to the other end. The leucocytes will be found almost entirely congregated at this distal end of the film. The films are allowed to dry in the air and are then ready for staining.

If the bacteria in question are Staphylococci, Pneumococci, Streptococci, Bac. Coli, etc., the film may be stained by Leishman's stain. The slide is laid in a Petri dish and covered with undiluted Leishman stain. This serves to fix the film. After one-half to one minute this is diluted with twice the volume of distilled water and the film is left in this for about two hours. (A shorter time will do, but the bacteria will not be so well stained.) It is then quickly washed with distilled water and dried with filter paper.

Another good method of staining such films is by Borrel's Carbol-Thionin. The film is fixed by placing for one minute in saturated solution of mercuric chloride; it is then washed and stained for two to five minutes with Carbol-Thionin and washed again.

For films containing tubercle bacilli special staining is necessary.

Fix in saturated solution of mercuric chloride. Stain with hot Ziehl-Nielsen carbol-fuchsin. Wash in water. Decolourise in 2½ per cent. sulphuric acid. Wash again. Dissolve up the haemoglobin of the red corpuscles with 4 per cent. acetic acid. Wash. Counter-stain with a very dilute solution of methylene blue, allowing it to act for only a few seconds. Then wash thoroughly.

To estimate the Opsonic Index, the number of bacteria in 100 consecutive leucocytes is counted and the average number per leucocyte determined ; this gives the "phagocytic index."

The phagocytic index of a normal serum, or of the "pooled" sera of a number of normal individuals, is similarly estimated.

The patient's opsonic index

$$=\frac{\text{patient's phagocytic index}}{\text{normal phagocytic index}}.$$

D. BACTERIOLYTIC TESTS.

THE PFEIFFER EXPERIMENT.

The Pfeiffer experiment has already been referred to and explained in the section on Bacteriolysins (page 39). As a practical test it has a two-fold application :

(*a*) In estimating the bacteriolytic power of a patient's serum ; and

(*b*) In the differentiation of certain bacteria.

(*a*) A notable increase in the bacteriolytic power of a patient's serum (as compared with normal serum) towards a certain bacterium always indicates a past or present infection with that bacterium. A bacteriolytic test may therefore help to clear up the diagnosis of certain diseases—chiefly cholera, typhoid fever, and para-typhoid fever. In these cases the bacteriolytic titer of the serum is usually only slightly raised during the course of the disease, and first reaches a high level during convalescence. The Pfeiffer experiment is therefore more suited for the diagnosis of past typhoid fever or cholera than for the diagnosis of the disease while still running its course.

If a patient is suspected of having had cholera the diagnosis may be made in the following manner:

Dilutions of the patient's serum are made with 20, 100 and 200 parts of bouillon. Of each dilution 1 c.c.m. is mixed with a platinum loopful of an 18-hour agar culture of virulent cholera vibrios, and injected into the peritoneal cavity of a guinea-pig of 250 g. weight.

A control guinea-pig receives one loopful of the same culture without serum, in 1 c.c.m. of bouillon.

If the reaction is positive after 20-60 minutes—*i.e.* if typical bacteriolysis is observed in hanging drop preparations made from the peritoneal exudate—then it may be stated that the patient supplying the serum has recovered from an attack of cholera. This information may be extremely important from the point of view of preventing the spread of the disease.

(*b*) *Pfeiffer Experiment for the differentiation of Bacteria.* This test is used chiefly for the diagnosis of suspected cholera vibrios.

An anticholera serum of a high bacteriolytic power is used and is injected, along with a loopful of the bacteria in question, into the peritoneal cavity of a guinea-pig. After twenty minutes at latest, typical bacteriolytic changes will be seen in the bacteria, if they are cholera vibrios.

The anticholera serum required for this test may be obtained from the Kaiserl. Inst. f. Infektionskrankheiten in Berlin.

E. COMPLEMENT-FIXATION.

If the reader has followed the sections on Bacteriolysis and Haemolysis (pp. 45 and 37), the complement-fixation experiment will be easily understood. It has there been explained that by haemolysis is meant the dissolution of the blood corpuscles, whereby their envelope is destroyed and their haemoglobin set free. In the serum of an animal immunised against red blood corpuscles, there exists an antibody (the " haemolysin "), which, acting in conjunction with complement, is able to produce the above effect when brought into contact with the particular kind of red corpuscles used for the immunisation.

If the serum containing the haemolysin (haemolytic serum) is heated to 56° C., and then mixed with the corresponding red corpuscles, no haemolysis occurs; but if a small quantity of complement (*e.g.* in the form of guinea-pig serum) is added to the mixture, it is very soon noted that the fluid in the tube is losing its thick turbid appearance and is becoming clear and transparent, but stained red with haemoglobin. That is to say, the corpuscles have been haemolysed by the united action of haemolysin and complement.

If, therefore, one is in possession of a haemolytic serum and of the corresponding red corpuscles, one has the means of ascertaining the presence or absence of complement in another fluid under observation. The fluid to be tested is simply added to this mixture of haemolytic serum and red corpuscles, and, if it contains complement, the corpuscles are lysed; if it does not they are left intact.

It will make the understanding of all complement-fixation experiments simple to the student if this mixture of haemolytic serum and red corpuscles is merely regarded as an "indicator"—in the same way as one regards a litmus or phenolphthalein solution in testing for the presence of a free acid or alkali. The haemolysin and red corpuscles combination is used in the complement-fixation experiments as a test for the presence or absence of free complement.

It has been proved by Bordet that while an antigen alone or an amboceptor alone will take up practically no complement, the combination of an antigen with its corresponding amboceptor *will* take up complement. It will be evident, then, that if, for example, an antigen is mixed with complement and the aforesaid test mixture of haemolysin plus red corpuscles is added to it, the corpuscles will be lysed, as the antigen itself has not been able to unite with the complement, and so has left it free in the mixture.

Similarly, if an amboceptor be mixed with complement and the test haemolysin plus red corpuscles be added, the corpuscles will be lysed for the same reason—that the complement is left ununited and at liberty to pass over to the amboceptor-antigen combination formed by the haemolysin and red corpuscles.

On the other hand, if a fluid containing both antigen and the corresponding amboceptor is mixed with complement, and the test mixture is subsequently added, there will be no lysis of the red corpuscles, as the complement is taken up by the united antigen and amboceptor and is not free to manifest its effect on the haemolysin and red corpuscles.

In these experiments it is, of course, necessary to observe accurately the quantitative conditions, as, obviously, if an excess of complement is present, it will not all be used up by the first antigen-amboceptor, and the remainder will pass to the haemolysin and red corpuscles (which, be it remembered, is likewise an antigen-amboceptor combination). A concrete example will make this clearer. If a rabbit is immunised against cholera vibrios, its serum will contain an amboceptor against these vibrios, *i.e.* an anticholera amboceptor. If this serum is collected and is heated for half an hour at 56° C., it will contain the amboceptor, but no complement. It will, therefore, be in a position to unite with cholera vibrios, and the combination of the two will be able to take up (adsorb) complement.

Thus, if an emulsion of cholera vibrios is mixed with some anti-cholera serum, and a small quantity of normal unheated guinea-pig serum is added to supply complement, it will be found that haemolysin and red corpuscles, subsequently added, remain unaffected—there is no complement free in the mixture, and thus the red corpuscles escape haemolysis. But if for the anti-cholera serum one substitutes normal serum, haemolysis does occur. The normal serum contains no amboceptors which can unite with the cholera vibrios; the complement, therefore, remains free, and when haemolysin and red corpuscles are subsequently added, it passes over to unite with them and lyse the red corpuscles.

This is the original Bordet-Gengou experiment—the ancestor of all complement-fixation tests. It ought not to be forgotten that for the complement-fixation test, which has now become so important in the diagnosis of syphilis ("Wassermann reaction"), we are so largely indebted to Bordet.

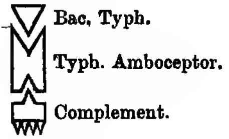
The complement-fixation test soon came to occupy the most important place in sero-diagnosis.

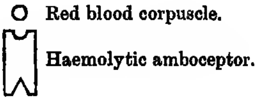
It may be employed:

(*a*) As a test for amboceptors; and
(*b*) As a test for bacteria.

(*a*) As a test for specific amboceptors it may be used, for example, in a case of suspected typhoid fever. An emulsion of known typhoid bacilli is mixed with some of the patient's serum (inactivated), and a small quantity of complement. This is put in the incubator at 37° C. for one hour and then the test fluid of haemolysin and red corpuscles is added. The mixture is again left for one hour at

37° C. At the end of this time, if the patient's serum contains typhoid amboceptors, there will be no haemolysis and the diagnosis of typhoid fever may be made. If, on the other hand, the serum contains no typhoid amboceptors, the complement will not be fixed and haemolysis will result.





The same test may be carried out with cholera vibrios and anti-cholera serum, with bacillus pestis and anti-plague serum, with Bordet's whooping cough bacillus and the serum of a whooping cough patient, and so on. The complement-fixation test has also been tried for the demonstration of antibodies in the serum of tuberculous animals and men, but so far the results are very unsatisfactory. Its outstanding importance as a practical test is due to its application in the diagnosis of syphilis.

Sero-diagnosis of Syphilis. The Wassermann Reaction. The scope of the complement-fixation test was greatly extended by the discovery of Wassermann and Bruck that not only emulsions of bacteria, but also bacterial extracts, can be used as antigen. Thus, in the case of micro-organisms which were not easily cultivated, extracts might be got from the various organs infected by them. These workers endeavoured to show the presence of anti-tuberculous amboceptors in the blood of tuberculous patients by using as antigen an extract of the diseased lungs, glands, etc. The experiments met with very little success in themselves, but they led on *Wassermann*, *Neisser*, and *Bruck* to try similarly if, in the serum of syphilitic patients, there are specific antibodies, the presence of which can be demonstrated by a complement-fixation experiment. As no method was known for cultivating the spirochaete pallida, in order to obtain an antigen they again had recourse to extracts of the diseased organs; this time, in contrast to their experience with tuberculosis, they met with signal success. They worked with watery extracts of syphilitic organs—especially extract of the liver of a syphilitic foetus. They regarded the process as an antigen-antibody reaction, and considered that a positive result indicated the presence of specific antibodies in syphilitic serum.

At first they found the reaction positive in only 19 per cent. of syphilitic cases.

Marie and *Levaditi* soon found that a watery extract of liver of a *normal* child could likewise be used as antigen; it gave complement-fixation with syphilitic serum or cerebro-spinal fluid from cases of general paralysis.

The next step was the discovery that alcoholic extracts of normal organs yielded an antigen as effective as the originally advised watery extract of syphilitic liver. This was a matter of much practical importance, as the difficulty of obtaining a suitable liver of a congenital syphilitic foetus was a stumbling-block in the way of many who wished to use the test.

Porges and *Meier* also reported good results, using an emulsion of lecithin as antigen.

These facts soon brought Levaditi, Wassermann and others to question whether the substances in the serum which gave the reaction were really antibodies after all. Since such varied substances were capable of acting as antigen, it was impossible to maintain that the process was a union of antigen and specific antibody, as had been supposed. Though the question cannot be regarded as definitely settled, the majority of the authorities now hold that the reaction is due to a physico-chemical change in the fluid brought about through the interaction of lipoids in the antigen with certain substances in the serum. This physico-chemical change manifests itself in the ability to adsorb complement.

The substance in the serum has been provisionally named the " Wassermann substance."

It has been shown that the reaction is not absolutely confined to syphilis ; it is found occasionally in malaria, relapsing fever, beri-beri, leprosy, eclampsia and scarlet fever. Only the last-named need be considered as likely to confuse the diagnosis in this country. Practically, however, there is no danger of such confusion ; in scarlet fever a positive Wassermann reaction is only an occasional and quite transitory phenomenon.

Technique of the Wassermann Reaction.

The method which will be described here is that employed by Dr. Boas in the Serum Institute, Copenhagen. This technique is strongly recommended as being the most accurate and trustworthy so far suggested, and as possessing the enormous advantage that it gives a quantitative result which is a guide to the effect of anti-syphilitic treatment.

The method of Boas cannot claim to be one of the "simplifications" which have been so eagerly clamoured for; it will probably be classed as "tedious" or "arduous" by those who aim at a "handy" method of diagnosing syphilis. The fact remains, however, that there is no "handy" method. The Wassermann reaction can only be performed, with any degree of safety, by a skilled laboratory worker using the utmost refinements of technique, and not omitting any of the controls. To give a diagnosis of syphilis from a Wassermann reaction, performed under other circumstances, is utterly unjustifiable.

On the other hand, when all the necessary precautions are observed, there is no diagnosis which the practitioner may make with more confidence than that of syphilis from a positive Wassermann test. The statistics of Boas will illustrate this. He examined the sera of 1064 cases of normal persons and of patients with the most diverse diseases (excluding syphilis), and only in one case did he obtain a positive reaction; this case was one of scarlet fever.

Of 269 cases of untreated secondary syphilis examined, 269 gave a positive reaction.

Of 63 cases of untreated tertiary syphilis, 63 gave a positive reaction.

For the carrying out of the test, the following are necessary :

1. The antigen.
2. The patient's serum.
3. Complement.
4. Red blood corpuscles.
5. Haemolytic amboceptor.

1. THE ANTIGEN.

The antigen originally advised by Wassermann was a watery extract of syphilitic organs. It is found, however, that alcoholic extracts of normal organs yield an almost equally reliable antigen, and, as these are much more easily procured, they will generally be employed.

The antigen used by Boas is that proposed by Michaelis—an alcoholic extract of human heart. The heart is obtained fresh from the post-mortem room ; it is freed from fat and pounded in a dry mortar with clean, sterilised, Bedfordshire river sand, in the proportion of one part of fat-free heart substance to nine parts of absolute alcohol. It is shaken for one hour at room temperature, filtered, and stored in a stoppered bottle.

Sachs has shown that the addition of cholesterin to the normal heart extract makes it much more effective as an antigen, and many workers now use this modification. To each 5 c.c.m. of alcoholic heart extract is added 0·4 c.c.m. of a 1 per cent. alcoholic solution of cholesterin (Kahlbaum).

The extract must be freshly prepared every few weeks, as it alters on keeping.

2. THE SERUM.

5-7 c.c. of blood are withdrawn by venipuncture from one of the veins in front of the elbow, and put into a sterile glass centrifuge tube. When the blood has clotted, it is separated all round from the sides of the tube, and is centrifugalised. The serum is poured off and must then be inactivated (*i.e.* heated in a water-bath at 56° C. for half an hour, to destroy the complement), and kept in the ice-chest till required.

Instead of using only one or two serum doses, as is done by other workers, Boas always employs at least five different doses: ·2, ·1, ·05, ·025, ·01 c.c.m. In this way the strength of the reaction can be judged, and the effect of anti-syphilitic treatment observed.

If a serum gives complete complement-fixation in the dose of ·1 c.c.m., one does not know whether this is the smallest fixing dose, or whether there might be fixation even with much smaller quantities. In examining cases during the course of treatment this is very important, as otherwise improvement in the condition may often be overlooked. For example, a serum before treatment might give complete fixation in the dose of ·01, and after treatment only in the dose of ·05. Doses above ·2 should not be used, as they may give rise to non-specific complement fixation.

3. THE COMPLEMENT.

Fresh guinea-pig serum supplies the complement. The vessels of the neck of the guinea-pig are cut across and the blood is collected in glass centrifuge tubes; the mouth of each tube is provided with a glass filter funnel to collect the blood without loss.

It is best to kill the guinea-pig on the day of the experiment, as the complement disappears when the serum is kept. The blood is allowed to clot and the serum then separated by centrifugalisation.

In the original Wassermann test the quantity of serum used for complement is always 0·1 c.c.m. Boas, however, finds that the amount of complement contained in the serum varies slightly, and that, in order to work with a constant complement dose, it is necessary to titrate the guinea-pig serum. The totally lysing dose of serum may vary from 0·07 to 0·04 c.c.m., but larger variations do not occur, as far as Boas has observed. In most cases the smallest totally lysing dose is 0·05 c.c.m. of serum, and when this is so Boas employs the usual Wassermann quantity of 0·1 c.c.m. of serum. If, however, 0·06 c.c.m. gives complete lysis he uses 0·12 c.c.m. (0·06 × 2). One can thus be sure of working always with the same complement dose—with twice the minimum completely lysing dose.

4. THE BLOOD CORPUSCLES.

The sheep's blood is run into a flask containing some large glass beads, and the glass is shaken in order to defibrinate it. After all the fibrin has adhered to the beads, the defibrinated blood is centrifugalised and then washed twice with normal saline solution. A 5 per cent. suspension of the corpuscles in saline solution[1] is then prepared (5 c.c.m.

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[1] Note: all the salt solutions used in these experiments must be accurately prepared—·85 per cent.—so as to be absolutely isotonic.

of the centrifugalised residue of corpuscles to 95 c.c.m. normal saline).

5. THE AMBOCEPTOR.

To obtain this, a rabbit is immunised against sheep's corpuscles. Sheep's blood is defibrinated, then centrifugalised and washed with saline solution to remove the serum ; it is again centrifugalised and again washed. The blood corpuscles are then injected into the peritoneum of the rabbit in increasing doses of 1, 2 and 4 c.c.m. at intervals of three to four days. Three days after the last injection the rabbit's blood is collected aseptically by fixing a canula into the carotid artery. The blood is defibrinated and centrifugalised. It is then put up in small tubes with aseptic precautions, and is inactivated (has its complement removed) by heating for half an hour at 56° C. in the water-bath.

It can be kept in the ice-chest for many months without losing any of its haemolytic power.

Before use the serum must be titrated. The titer of a serum produced in this way is frequently about 1:2500—that is to say, 1 c.c.m. of a 1 : 2500 dilution of this serum will cause complete haemolysis in two hours of 1 c.c.m. of a 5 per cent. suspension of sheep's corpuscles, in presence of 1 c.c.m. of complement diluted 1 : 10.

The quantity of amboceptor used in the Boas experiment is two-and-a-half times the totally lysing dose. Other workers use twice the totally lysing dose and others again use three or four times that dose. It is, at all events, necessary to have an excess of amboceptor present.

The carrying out of the reaction. Three preliminary experiments must be done :

(1) The titration of the amboceptor ;
(2) The titration of the complement ; and
(3) The titration of the antigen.

(1) *Titration of the Amboceptor.* A number of dilutions of the rabbit's serum (called haemolytic serum, haemolysin or simply amboceptor) are prepared, *e.g.* 1 : 500, 1 : 750, 1 : 1000, 1 : 1500, 1 : 2000, 1 : 2500, etc. The dilutions are made with normal saline solution. A series of small tubes is then arranged in a stand, and each tube is marked to correspond with one of these dilutions ; each tube then gets 1 c.c.m. of its appropriate dilution. 1 c.c.m. of complement (1 : 10 dilution) is added to each tube, then 1 c.c.m. 5 per cent. blood corpuscles, and 2 c.c.m. normal saline to bring the quantity in each tube to 5 c.c.m. The tubes are shaken and put for two hours in the incubator at 37° C. The smallest dose of amboceptor which causes complete haemolysis gives the so-called " titer " of the serum. For example, if haemolysis is complete with the dilution of 1 : 2000 and incomplete with 1 : 2500, the titer is 1 : 2000, and the dose to be used in the subsequent reaction is two-and-a-half times the amount represented by 1 c.c.m. of this dilution—*i.e.* 1 c.c.m. of the dilution 1 : 800.

(2) *Titration of the Complement.* One sets out a series of five tubes containing respectively 0·07, 0·06, 0·05, 0·04, and 0·03 c.c.m. of the guinea-pig serum made up to 1 c.c.m. with normal saline solution. To each tube is added 1 c.c.m. of the amboceptor dilution,

just determined (two-and-a-half times the minimum totally lysing dose). 1 c.c.m. of a 5 per cent. suspension of sheep's corpuscles and 2 c.c.m. of saline solution are then added to each tube.

The tubes are put in the incubator at 37° C. for two hours. If one finds, for example, that 0·05 c.c.m. is the smallest totally lysing dose, one must use, for the main experiment, twice that amount = 0·1 c.c.m.

(3) *Titration of the Antigen.* A series of six tubes is set out containing respectively ·4, ·3, ·2, ·15, ·1, ·075 c.c.m. of antigen, made up to 1 c.c.m. with normal saline solution. 1 c.c.m. of 1 : 10 complement dilution is added to each tube and 1 c.c.m. saline solution. These tubes are incubated for one hour at 37° C. and 1 c.c.m. sheep's blood corpuscles (5 per cent. emulsion), and 1 c.c.m. haemolysin dilution are added. At the end of an hour and a half the result is read off. The largest dose which causes no inhibition of haemolysis is noted, and half this quantity is used as the antigen dose in the main experiment which follows.

These preliminaries over, one proceeds to the main experiment (" Hauptversuch ").

As has already been mentioned the serum to be examined is tested in five different doses :

·2 c.c.m. in tube 1.
·1 ,, ,, 2.
·05 ,, ,, 3.
·025 ,, ,, 4.
·01 ,, ,, 5.
·2 ,, ,, 6 (serum control).

Each tube then receives sufficient saline (·85 per cent.) to bring the volume up to 1 c.c.m. 1 c.c.m. of a 1 : 10 dilution of guinea-pig serum is then given to each tube. The antigen is added finally to the tubes 1-5; if the dose which causes complete inhibition of haemolysis be 0·4 c.c.m., half that quantity = 0·2 c.c.m. is used and saline added to make up to 1 c.c.m. It is best to put the requisite ,quantity of saline into the tube first, and then let the antigen run gently down the side of the tube and shake thoroughly. Tube 6 receives no antigen as its object is to show that ·2 c.c.m. of the serum does not of itself prevent haemolysis. In place of the antigen it receives 1 c.c.m. of saline solution.

The tubes are all thoroughly shaken and then placed for one hour in the incubator at 37° C. This length of time is necessary in order to give ample opportunity for the fixation of the complement, and to avoid missing the weaker reactions.

At the end of the hour, 1 c.c.m. of the suitable haemolysin dilution (already determined) is added to each tube, and 1 c.c.m. of a 5 per cent. suspension of sheep's blood corpuscles.

The tubes are again shaken and are replaced in the incubator for two hours at 37° C. At the end of this time they are taken out and put to stand overnight in the ice-chest. Next day the results are read off. In the tubes which give a completely positive reaction (total fixation) all the corpuscles are found at the bottom of the tube, leaving the fluid above clear and colourless; in the tubes which give a completely negative reaction, on the other hand, no corpuscles are found at the bottom of the tube, and the fluid is deeply stained with haemoglobin. Between these two results—no haemolysis and complete haemolysis—all grades are found; the reaction may be strongly but not completely positive, in which case most of the corpuscles lie at the bottom of the tube, but a few are lysed and give to the supernatant fluid a slightly red colour; or it may be less strongly positive, in which case fewer corpuscles are found at the bottom, and the fluid above is of a deeper red; or it may be almost, but not altogether, negative, in which case a few corpuscles are found at the bottom of the tube which contains a deeply haemoglobin-stained fluid.

The reaction is positive if the first tube shows no haemolysis, or only partial haemolysis. A very slight degree of fixation should not, however, be counted as a positive reaction.

The strength of the reaction may be indicated by the use of signs as follows:

Strongly positive	XXXXX	Inhibition of haemolysis in 5 tubes.
	XXXX	„ „ „ „ 4 „
	XXX	„ „ „ „ 3 „
Weakly positive	XX	„ „ „ „ 2 „
	X	„ „ „ „ 1 tube
Doubtful	÷	Slight inhibition in 1 or more tubes.
Negative	–	Complete haemolysis in all tubes.

Boas uses a haemoglobin scale, a comparison with which indicates the exact amount of haemolysis which has taken place in each tube. (See Boas, *Die Wassermannsche Reaktion*, page 30.)

In carrying out any complement-fixation test, numerous controls are necessary to avoid the sources of error which surround the experiment.

(1) THE ANTIGEN CONTROL.

A tube must be put up, containing antigen, complement, haemolysin and corpuscles, but not containing any serum. This is to test whether the antigen alone is able to prevent haemolysis.

It is customary, after titrating the antigen and finding the largest dose which allows a complete haemolysis, to work with half that dose in the subsequent tests.

In the case of the alcoholic heart extract used by Boas, this dose is too small, and, if it is used, many definitely positive reactions will be overlooked. Boas therefore works with an antigen dose which in itself allows complete haemolysis, but which is greater than half the non-fixing (" unterhemmend ") dose. If, however, one is working with a watery syphilitic extract as antigen, it is necessary to limit the amount used to half the non-fixing dose.

No. 1 control tube therefore contains :

Antigen (dose used in main experiment or twice that dose made up to 1 c.c.m. with normal saline).
Complement (·1 c.c.m. made up to 1 c.c.m. with saline).
Haemolysin (1 c.c.m. of the appropriate dilution, already determined).
Sheep's corpuscles (1 c.c.m. of the 5 per cent. suspension).
Saline (1 c.c.m., to take the place of the serum which is omitted).

(2) THE SERUM CONTROL.

The serum alone may cause complement-fixation if used in a sufficiently large dose. It is therefore necessary to put up along with each serum a control tube containing the largest dose of the serum which is used in the test; in this tube haemolysis must be complete.

No. 2 Control Tube therefore contains:

Serum (·2 c.c.m. made up to 1 c.c.m. with saline).
Complement (0·1 c.c.m. made up to 1 c.c.m. with saline).
Haemolysin (1 c.c.m. of appropriate dilution).
Corpuscles (1 c.c.m. 5 per cent. suspension).
Saline (1 c.c.m., in place of antigen).

This control tube accompanies each serum; thus, if one is testing twelve sera, there will be twelve serum control tubes.

(3) A CONTROL POSITIVE AND

(4) A CONTROL NEGATIVE SERUM are always tested alongside each series. These must be known cases, the former definitely syphilitic, and the latter non-syphilitic, and must have given on a previous examination a positive and negative reaction respectively.

(5) THE HAEMOLYTIC SYSTEM CONTROL.

This tube is to show that the haemolytic system is effective and properly arranged.

No. 5 control tube will contain:

Complement (·1 c.c.m. made up to 1 c.c.m. with saline).

Haemolysin (0·5 c.c.m. of the dilution used in the main experiment plus 0·5 c.c.m. saline).
Corpuscles (1 c.c.m. 5 per cent. suspension).
Saline (2 c.c.m.).

The following shows at a glance the arrangement and contents of the tubes necessary for testing one specimen of serum :

Let it be assumed that ·2 c.c.m. of antigen represents the non-fixing dose or half the non-fixing dose, as already explained ; that the complement titer is ·05 and that double that quantity therefore is employed ; that the titer of the amboceptor is ·0006, and that two-and-a-half times that amount is used ; and that the sheep's blood corpuscles are made up in a 5 per cent. suspension.

Enough saline must be added to bring the quantity of each of the five ingredients up to 1 c.c.m. ; each tube therefore contains 5 c.c.m.

	1.	2.	3.	4.	5.	6.
Antigen,	·2 c.c.m.	·2	·2	·2	·2	
Serum,	·2	·1	·05	·025	·01	·2
Complement,	·1	·1	·1	·1	·1	·1
Amboceptor,	·0015	·0015	·0015	·0015	·0015	·0015
Corpuscles,	1 c.c.	1 c.c.	1 c.c.	1 c.c.	1 c.c.	1 c.c.

Tube 6 represents the serum control.

Numerous modifications of the Wassermann reaction have been proposed, but none so far have given thoroughly satisfactory results, and the majority have proved utterly untrustworthy.

Bauer uses no rabbit-sheep amboceptor, but takes advantage of the haemolytic amboceptor for sheep's corpuscles which is normally present in small amount in human blood.

Hecht employs, like Bauer, the normal haemolysin of the human serum, and he also adds no guinea-pig complement, but uses instead the complement in the human serum under examination. This necessitates, of course, that the serum shall not be inactivated, and it has been abundantly proved that the use of such " active " sera gives rise to numerous non-specific reactions.

Noguchi uses human blood corpuscles and the haemolytic serum of a rabbit which has been immunised against human blood.

V. Dungern uses complement dried on blotting paper. His method, which is thus performed with old, dried and weakened complement, and in which all the controls are omitted, is obviously quite unreliable.

The method which is used in the sero-diagnostic service of the Pasteur Institute, Paris, is a combination of the Bauer and Hecht modifications. The technique is simple and time-saving, and will therefore be described, though for accurate and trustworthy results it cannot rank with the method of Boas, described above.

BAUER-HECHT MODIFICATION.

The complement and amboceptor are supplied by the patient's serum, which therefore must not be inactivated.

The antigen is an alcoholic extract of the liver of a congenital syphilitic foetus. The liver is ground down, dried "in vacuo" over sulphuric acid, then reduced to powder. Some of this powder is suspended in absolute alcohol and macerated for several hours; the fluid is then decanted. A dilution of 1 : 10 in saline solution is made, and this dilution is used in the experiments. The antigen is titrated with a normal human serum.

For each serum to be tested, four small tubes are set out, as follows :

Tube No. 1.	No. 2.	No. 3.	No. 4 (Control).
Fresh serum, 0·1 c.c.m.	0·1	0·1	0·1
Antigen, 0·1 c.c.m.	0·2	0·3	—
Saline Solution, 0·2 c.c.m.	0·1	—	0·3

These are put in the incubator at 37° C. for 1½ hour, then to each tube is added 0·1 c.c.m. of a 5 per cent. suspension of sheep's corpuscles.

The reaction is considered positive where there is no haemolysis, or almost none, in the first three tubes and complete haemolysis in tube 4.

The patient's serum must be used as fresh as possible, as the complement soon becomes weakened. If the serum has thus become weak in complement, or if it contains too small an amount of amboceptor —a condition which is shown by the haemolysis in tube 4 being incomplete—it is necessary to add 0·1 c.c.m. of fresh normal human serum to all four tubes, to supply the defect.

(*b*) The complement-fixation test has also been used in the diagnosis of bacteria; in this case it is necessary to have serum of an animal immunised against the bacteria in question. For example, *Schutze* has endeavoured to differentiate genuine cholera vibrios from the cholera-like bacteria by mixing an extract of the vibrios in question with the serum of a rabbit immunised against true cholera; complement is then added, and after one hour in the incubator at 37° C., the mixture has the further addition of haemolysin and red corpuscles. If the bacteria are genuine cholera vibrios, complement-fixation will occur, and haemolysis will not take place. It must be added, however, that these experiments were not quite successful in differentiating the bacteria.

Bordet has also used this test to prove that his "bacillus pertussis" is the true cause of whooping cough.

More recently *Dean* has quite successfully differentiated between various members of the typhoid and paratyphoid groups of bacteria, by using the complement-fixation method. He found it necessary to work with very high dilutions of both antigen and anti-serum.

F. TUBERCULIN DIAGNOSTIC TESTS.

There are several different reactions which come under this heading:

(1) The original tuberculin reaction of Koch.

(2) The skin reaction of V. Pirquet.

(3) The ophthalmo-reaction of Calmette, and
(4) The opsonic reaction, after diagnostic tuberculin injection, of Wright.

(1) Koch's method is founded on the fact that a tuberculous guinea-pig is made profoundly ill or killed by a dose of tuberculin which has no effect on a non-tuberculous guinea-pig. An analogous phenomenon is found in man—a tuberculous person reacts to the subcutaneous injection of a small dose of tuberculin (*e.g.* 1 mg.) which produces no symptoms at all in a non-tuberculous person. Obviously in human practice only small doses, incapable of causing injury to a tuberculous person, are applicable. With these small doses, however, Koch claims that the diagnosis of tuberculosis can be made clear.

WHAT IS OLD TUBERCULIN ?

It is a brownish, slightly viscous fluid, produced by growing tubercle bacilli in glycerinated bouillon for 4-5 weeks, then sterilising in a current of steam for half an hour, evaporating the fluid to one-tenth of its volume at a temperature of 70° C., and filtering. The filtrate is the " old tuberculin."

Now it will be obvious that it is rather difficult to say exactly what this substance is. It contains, of course, substances secreted and excreted by the tubercle bacillus whilst growing in the bouillon ; it also contains disintegration products of dead tubercle bacilli. But besides these it contains substances extracted from the bacilli under the influence of heat, and altered products of the glycerine, peptone, etc., composing the medium. For injection, dilutions of this are freshly prepared in sterile saline solution.

1 c.c.m. Old Tuberculin is reckoned as =1000 mg.
·1 ,, ,, ,, ,, =100 mg.
·001 ,, ,, ,, ,, =1 mg.

Clinical Employment.

The occasional untoward results obtained by the therapeutic use of large doses of old tuberculin have made practitioners chary of injecting it into the human subject. It seems, however, to have been abundantly proved that if one works with small doses, such as those required for diagnostic purposes, and observes the contra-indications, there is no danger of harming the patient whilst making the diagnosis.

Loewenstein, who has carried out 20,000 injections, has never seen any serious disturbance follow.

Contra-indications are: fever, haemoptysis, heart and kidney troubles, diabetes and epilepsy.

The injection is made subcutaneously at the angle of the scapula or in the flank.

As a rule the dose is 1 mg. for the first injection; in very debilitated patients, however, and in children it should be ·1 mg.

The patient's temperature must be closely observed for some days preceding the injection and then every four hours after. If the temperature has been quite steady before the injection, a rise of even half a degree above the highest point preceding the injection, is regarded as a positive reaction.

If there is no rise of temperature, the injection is repeated after two or three days, but this time 5 mg. are injected. If an insignificant rise of temperature has taken place, the dose is not increased, but the original amount is repeated and, if the patient is tuberculous, the reaction will then be obtained.

Besides the fever in a tuberculous patient there will be other symptoms which indicate a positive reaction—headache, shivering, pains in the back and limbs, etc. There is also redness and swelling at the site of injection, which are absent in non-tuberculous cases.

In the case of laryngeal tuberculosis or lupus, one observes also increased redness and swelling of the diseased parts.

(2) THE SKIN REACTION OF V. PIRQUET.

V. Pirquet noted that if old tuberculin is introduced into the skin of a tuberculous patient, a papule appears at the spot, whilst in non-tuberculous subjects no such reaction occurs.

Technique. Cleanse the skin of the inner side of the forearm with camphor carbolic and then with ether. Place two separate drops of old tuberculin at spots on the skin about four inches apart. Scarify the skin at a spot between the drops (for control), and then under each drop, by means of an ordinary vaccination perforator. A few strands of cotton wool are left for about ten minutes in each drop to prevent it flowing off.

Almost immediately a certain amount of reaction is seen at all three scarified spots; this is merely traumatic and passes off in a few hours. The specific reaction commences, at the earliest, from three to twenty-four hours from the time of scarification. It is characterised by the appearance of a small red papule, which rapidly increases to a size of about 10-30 mm. Sometimes the papule, though well raised, lacks the red colour, and can only be made out by touch or by observation from the side. The reaction is at a height generally within forty-eight hours, and the papule then begins to fade.

Clinical Value of the Reaction. It has been proved that in adults the reaction gives unreliable results, as it is positive in 70 per cent. of all cases. In children, however, it is of some value. It is present only in a small percentage of clinically non-tuberculous children, and so it has much more significance here than in adults.

The method is safe and simple.

Moro's Dermo-reaction. Moro found that by rubbing a tuberculin ointment into the skin in tuberculous patients a reaction is obtained which consists of small papules on an erythematous skin surface.

The ointment is made of old tuberculin and lanoline (anhyd.), equal parts.

So far there is no concensus of opinion as to the value of the reaction.

(3) THE OPHTHALMO-REACTION OF CALMETTE.

Opinions still differ on the question of the specificity of this test and the dangers attending its use. It is certain that the method originally recommended by Calmette gave rise frequently to very serious disturbance in the eye, and necessitated a risk to the patient which was not permissible. Recently the strength of the solution used has been reduced to one hundredth part of that originally advised, and it is claimed as being free from risk.

Wolff-Eisner was the first to point out that the conjunctiva of a tuberculous person is hypersensitive to tuberculin. Calmette then used this hypersensibility for the purposes of diagnosis.

A few drops of a solution of tuberculin are instilled into the eye of the patient ; the other eye is the control and is untreated. In tuberculous patients, within a few hours, redness and lachrymation are noticed in the treated eye. After from twelve to twenty-four hours the conjunctiva appears intensely red, the caruncle is red and swollen, and frequently there is some exudation. These symptoms then gradually subside.

The solution recommended is prepared from glycerine-free desiccated tuberculin. It should contain ·018 of the desiccated tuberculin in 100 parts water. One or two drops are instilled into the eye with a pipette ; the lids are kept open till the drops diffuse.

Contra-indications. The Calmette reaction is contra-indicated in all eye diseases of whatever origin.

Citron recommends 1 per cent. solution of old tuberculin. He claims that the reaction is thus very mild and extraordinarily specific. He insists on the use of none but the Meister, Lucius and Brüning preparation for this reaction. The dilution must be made freshly for the experiment with sterile water or saline solution.

One drop is instilled into the eye.

(4) The Opsonic Reaction after injections of Tuberculin (T.R.).

Wright found that the injection of tuberculin (T.R.) into tuberculous patients caused a rise in the opsonic index, and that this was not the case with non-tuberculous persons. By estimating the opsonic index before and after an injection of tuberculin (T.R.), therefore, he claimed to obtain a guide to diagnosis.

This question is still undecided.

G. SERO-DIAGNOSIS OF MALIGNANT TUMOURS—MEIOSTAGMIN REACTION.

An extraordinary number of immunity reactions have been employed in the attempts to find a diagnostic test for cancer. In every instance, however, the results have been disappointing, and none of the tests—unless perhaps it be the Ascoli Meiostagmin reaction—can so far claim the serious attention of the clinician.

The Meiostagmin reaction is founded on the fact noted by Ascoli that by the coming together of an antigen and the corresponding antibody there results a reduction of the surface tension in the liquid containing these. This reduced surface tension causes the liquid to form a greater number of drops for a given quantity; the number of drops can be estimated by means of an instrument known as Traube's Stalagmometer.

The Meiostagmin reaction is seen in typhoid fever, tuberculosis, syphilis, echinococcus, etc. Its chief interest, however, is in relation to the diagnosis of malignant tumours.

Technique. The chief difficulty is in the preparation of the antigen, as this necessitates a somewhat tedious process and is extremely unstable when made. Ascoli has tried many methods in his endeavour to simplify the experiment; the most recent is the following:

Non-degenerated portions of malignant tumour (cancer or sarcoma) are cut down into small pieces, and this is spread out in a thin layer on glass plates and kept at a temperature of 37° to dry, or is dried *in vacuo*. The dried substance is then pulverised and the powder extracted with methyl alcohol (in the proportion of 5 g. to 25 c.c.m.) for twenty-four hours at 50° C. in closed vessels, being occasionally shaken. While still hot it is filtered; the filtrate is allowed to cool and then again filtered through Schleicher and Schull's paper, No. 590.

The next step is the titration of the antigen, to find in what dilution it should be employed. A normal serum diluted to 1 : 10 with normal saline solution is used and 9 c.c.m. of this are mixed with 1 c.c.m. of various antigen dilutions (1 : 50, 1 : 100, 1 : 150, 1 : 200, etc.). The antigen dilutions are made with distilled water. Another tube is put up containing 9 c.c.m. of the diluted serum and 1 c.c.m. of distilled water.

The tubes are put for two hours at 37° C. and the drop number for each fluid is then estimated by Traube's stalagmometer. The antigen is to be chosen in the dilution which does not alter the drop number for normal serum by more than 1 drop—the strongest dilution which fulfils this condition being chosen.

The actual experiment is done as follows :

The drop number of the serum to be tested, diluted to 1 : 10 with saline solution, is first estimated with the stalagmometer. Then two tubes are taken and one receives 9 c.c.m. of diluted serum plus 1 c.c.m. antigen dilution, whilst the other receives 9 c.c.m. diluted serum plus 1 c.c.m. distilled water.

These tubes are put in the incubator at 37° C. for two hours or in the water-bath at 50° C. for one hour. At the end of this time they are removed and allowed to cool and the drop number of each is estimated.

Alterations of the number above two drops (as compared with the control containing distilled water instead of antigen) are regarded as positive reactions.

In each series of tests there should always be a known negative and a known positive serum as controls, to show that the antigen has not altered.

Practical Value. This test has been very little used in this country, chiefly owing to the difficulty of preparing a reliable antigen. The results obtained by workers abroad, however, have been very encouraging.

Ascoli and Izar, in 100 cases of malignant tumour, got 93 positive reactions ; in 103 cases of other disease they got only 1 positive reaction. Tedesko, Stabilini and others report equally favourable results, and quite recently Leitch, in this country, has been able to report some findings which are distinctly hopeful. The value of the reaction, however, is still *sub judice.* The question of its rationale is also still quite vague ; to call it an antigen-antibody reaction is mere speculation. What substances are extracted from the tumour by the process described are quite uncertain, and the mode of their action on the serum is equally unknown. But the fact that the operation is quite empirical will not be sufficient to condemn it in the eyes of the clinician who still eagerly awaits any test that will aid in the early diagnosis of malignant disease.

CHAPTER VII.

ANAPHYLAXIS.

(Fr. *Anaphylaxie*; Ger. *Ueberempfindlichkeit.*)

ANAPHYLAXIS is the name given to a condition of abnormal sensitiveness of an animal to the action of certain substances. These substances may be foreign albumens of any kind, *e.g.* blood serum of an animal of a different species, egg albumen, milk, extracts of various organs, extracts of bacteria, etc.

If, for example, a small quantity of horse serum, say $\frac{1}{100}$th c.c.m., is injected into a guinea-pig, it renders the guinea-pig hypersensitive to horse serum. This hypersensitiveness passes quite unnoticed unless, after a certain interval, the guinea-pig is injected a second time with horse serum, when the anaphylactic condition shows itself in severe symptoms, perhaps resulting in death in a few minutes.

The discovery of this phenomenon called forth much surprise and incredulity, as scientists had for so long been accustomed to regard the reaction of immunisation, or diminished sensitiveness, as the appropriate response of an organism to the injection of foreign substances.

Though it is only a few years since this condition of Anaphylaxis was first studied, it has now become the subject of one of the largest sections of Immunity research. The amount of work done on Anaphylaxis has been enormous, and the volume of its literature is growing every day. It is a subject of the greatest importance to every student of Immunity, not only on account of its practical bearing on serum therapeutics, but because, as a problem, it holds in its depths the secret of many of the vital questions of Immunity, and because the research work now being done on anaphylactic phenomena promises to do more than any other contemporary research, for the elucidation of these questions.

We shall consider the matter under three headings :

I. Facts known regarding anaphylactic phenomena ;
II. Theories ; and
III. Practical application in medicine.

I. FACTS KNOWN REGARDING ANAPHYLAXIS.

The first to observe this phenomenon was *Ch. Richet.* When experimenting with extracts of the tentacles of certain sea-anemones, he found that dogs, which had received one injection of the extract, became unduly susceptible to the action of a second dose ; such dogs could be killed by a quantity which represented only a fraction of the fatal dose for an untreated dog. To this condition of abnormal susceptibility, he gave the name "Anaphylaxis."

Later *Arthus* observed similar phenomena with horse serum. He noted that rabbits, which have received preliminary injections of horse serum, become unusually sensitive, so that a subsequent injection may cause them very serious symptoms or may even produce death. He could similarly bring about a condition of anaphylaxis against milk by giving an animal preliminary injections of milk.

He showed that the anaphylaxis was *specific*—a preliminary injection of serum rendered the animals hypersensitive to serum but not to milk or other albumen ; a preliminary injection of milk rendered them hypersensitive to milk, but not to serum, and so on.

Theobald Smith made observations on the sensitising effect of horse serum, which led along the same lines as the observations just quoted. He had observed that guinea-pigs which were used by manufacturers of antitoxin in estimating the strength of the serum (and which consequently had received injections of small doses of horse serum) were thereby rendered hypersensitive to normal horse serum. A small dose of serum given to such a guinea-pig might cause its death almost instantly.

The astonishing fact here is that ordinarily innocent substances, like normal serum or milk, can behave so viciously. And the further astonishing fact is that, whereas one had previously been in the habit of considering that the reaction of an animal to an injection was one of immunisation, (whereby the animal was rendered *less* sensitive to a subsequent dose of the same substance) one now found that exactly the opposite event might occur. Here, as Besredka remarks, the rules of Immunity are " standing on their heads ! "

About the same time, *V. Pirquet* and *Schick* published observations on certain symptoms occurring in children treated with antidiphtheritic or antiscarletinal serum, symptoms which, in their general features, correspond with those described in hypersensitised animals. (See further under "Serum disease.")

The work of *Otto* and of *Rosenau* and *Anderson* has helped greatly to define these phenomena.

It was found that, in order to produce hypersensibility in a guinea-pig against horse serum, a very small dose must be used; $\frac{1}{100}$th c.c.m. subcutaneously or intraperitoneally is a common dose, but much smaller quantities (even as little as $\frac{1}{100,000}$th c.c.m.) may suffice.

After this preparatory or "sensitising" injection there occurs an incubation period before the anaphylactic condition becomes demonstrable. With the above dose, this incubation period usually lasts eight to twelve days. With larger doses, *e.g.* 4-5 c.c.m. intraperitoneally, the anaphylactic state may likewise be produced, but in this case the incubation period is much longer—lasting even from three to four months. If no further serum is injected, this condition of anaphylaxis quite escapes observation. In order to bring it into evidence, a second injection must be given after the expiry of the incubation period. For this second, or "test" injection, a much larger dose is required than for the sensitising injection; 5 c.c.m. intraperitoneally or $\frac{1}{4}$ c.c.m. intracerebrally is a common dose.

The symptoms may come on almost immediately after this injection; the animal becomes very restless, there is severe dyspnoea, there may be convulsions or paralysis; these symptoms are accompanied by rapid fall in the blood pressure, and the animal may die within a few minutes.

Anti-anaphylaxis. If, on the other hand, a second fairly large dose is given *before* the end of the incubation period, the animal is de-sensitised or rendered immune to anaphylaxis. It is then said to be in a condition of anti-anaphylaxis or de-sensitisation. A test dose may now be given after the expiry of the twelve days' incubation, without producing any symptom.

Passive Anaphylaxis. Just as a condition of passive immunity may be conferred on an animal by giving it an antitoxic serum from an actively immunised animal, so a condition of passive anaphylaxis may be conferred on an animal by giving it an injection of serum from another which has been actively sensitised. This fact is used as a strong argument in favour of the view that in anaphylaxis, as in immunity, antibodies appear in the blood, and may be transferred in this medium to another animal. This passive anaphylaxis, according to most observers, does not appear immediately after the serum is injected, but generally only after an interval of about twenty-four hours. It is thought that during this period the antibody is fixing itself to the nerve cells and rendering them unduly sensitive. Richet, however, holds that the passive anaphylaxis may be demonstrated immediately.

Anaphylaxis produced by injections of bacteria and bacterial extracts. Since this phenomenon of hypersensibility is of a general nature and shows itself after the injection of any kind of alien albumin, it is not surprising that it should also occur after the injection of extracts of bacteria, which, of course, contain vegetable albumins. *Wolff-Eisner* was the first to show that bacterial proteins are capable of giving rise to anaphylaxis.

Then *Rosenau* and *Anderson* showed that animals treated with cultures of B. coli., B. tuberculosis, B. subtilis, etc., after an incubation period of at least eleven days, were sensitive to doses which did not affect untreated animals.

Besredka emphasises the fact that only *living* cultures of bacteria produce these effects; killed cultures do not sensitise.

Friedberger, on the contrary, finds that dead cultures are even more suitable for the production of the sensitising substance (called by him "Anaphylatoxin").

II. ANAPHYLAXIS AS A FACTOR IN PRACTICAL MEDICINE.

If we confine ourselves to what is definitely known regarding the evidences of anaphylaxis in man, and neglect the alluring vistas of theoretical possibilities, we shall find that the horizon is not wide.

The question of *Serum-Anaphylaxis* in man has come to be important owing to the extensive use of serum therapy. The question of *Bacterial Anaphylaxis*, interesting from the theoretical point of view, has not, so far, claimed much practical significance. The question of "*Natural Anaphylaxis*" arises in connection with the idiosyncrasies shown by some people towards certain food stuffs—*e.g.* egg, pork, crab, mussels, shrimps, strawberries, etc. The cases where these generally innocuous substances produce severe symptoms, when eaten in very small quantities, may perhaps be instances of "*Natural Anaphylaxis.*" This problem is still unsolved. But it is undeniable that the symptoms of urticaria, acute asthma, swelling of tongue and uvula, and other angio-neurotic disturbances, seen in certain persons after the ingestion of these foods, have a close resemblance to anaphylactic phenomena.

The Serum Disease ("Serum Krankheit"; "Maladie Sérique").

This is a condition of hypersensibility occasionally evinced in man after the therapeutic injection of serum. It rarely occurs after a single injection, and when it does occur, its symptoms are never severe. After a second serum injection, however, symptoms are not uncommon—in France they occur in about 14 per cent. of the cases so injected.

The symptoms may come on in from four to six hours or after an incubation period of from five to fifteen days from the time of injection.

The early symptoms are:

Oedema at the point of injection, which may be severe and extend over nearly one-half of the abdomen;

Nausea and Vomiting, *Dyspnoea*, *Muscular and Articular Pains*, *Fever*, and a *sensation of fatigue and depression.*

The late symptoms—occurring after the fifth day—consist in:

Eruptions, which are chiefly of an urticarial nature and accompanied by pruritus;

Inflammation of Mucous Membranes—laryngitis, enteritis, etc.;

Pains in Muscles and Joints;

Fever and General Malaise;

Swelling of the Glands.

Fortunately the symptoms of the serum disease are generally but trivial, and they do not in any degree limit the use of serum therapy. It is doubted by some authorities whether a single death has ever resulted; the fatal cases (about twenty in number) reported as due to the serum disease, have almost invariably been attributable to other causes. There are, however, one or two well-authenticated instances of death, where one can hardly doubt that anaphylaxis was the cause. In any case, there is no danger of producing symptoms by giving repeated serum injections at a few days' interval in the course of a disease. Even in the administration of anti-meningococcic serum, where the injections have frequently to be repeated over a number of weeks, no serious or lasting injury is ever seen to result from the anaphylactic symptoms which often appear. It is only when an interval of several weeks or months occurs between the injections that there is a risk of alarming symptoms of anaphylaxis. It should also be remembered that the severe symptoms of anaphylactic shock seen in sensitised animals treated with comparatively small doses, come on almost invariably after intravenous or intracerebral injections. With the subcutaneous method—that almost invariably employed in man—the effect is infinitely more mild.

For the avoidance of these accidents various measures have been tried. It has been noted that serum is much more toxic when fresh; no serum should be used for therapeutic purposes which is less than two months old.

Netter advises giving the patient doses of calcium salts; children receive one gramme of calcium chloride on each of three days.

Wright advises calcium chloride as a routine after serum injections, especially prophylactic injections. The dose recommended for an adult is 30 grains, chloride or lactate, daily from the sixth till the tenth day after the injection.

Doubtless we shall yet discover a method of desensitising the human being, as one does the guinea-pig, by giving a second injection shortly after the first. This, however, is not yet demonstrated.

The risk of anaphylactic symptoms may also be avoided by using for the second injection the serum of a different species of animal—*e.g.* the goat, instead of the horse.

Other manifestations of what are considered anaphylactic phenomena occurring in man are seen in hay-fever,[1] certain forms of asthma,[2] and certain cases of puerperal eclampsia.

[1] *Hay-fever.* See under Serum Therapy, page 85.

[2] A number of cases of asthma are cured or at least freed of their asthma for a year or two after the injection of antidiphtheritic serum. Are they made anti-anaphylactic by this dose?

Other examples of anaphylactic symptoms in man are probably to be found in the tuberculin reaction, the reaction after small-pox re-vaccination, and the syphilis re-inoculation reaction.

The Tuberculin reaction. It is not yet definitely proved that this is an ordinary anaphylactic phenomenon; many leading authorities, however, believe it to be so.

Reaction to Small-pox re-vaccination. The pustule in a re-vaccination comes on after a shorter incubation period than in a primary vaccination; it is also surrounded by an aureola of acute inflammation, and it runs its course much more rapidly—all things pointing to increased sensibility.

Syphilis re-inoculation. Landsteiner has shown that syphilitic patients react to a subsequent introduction of syphilitic virus with symptoms which have the features of anaphylaxis.

III. THEORIES OF ANAPHYLAXIS.

Numerous theories have been offered to explain this curious phenomenon. Certain authors have explained the symptoms as due to the presence of precipitins in the blood of the sensitised individual. These would cause precipitation of the subsequently injected serum and the consequent formation of tiny thrombi which, by blocking up the vessels, would give rise to the various symptoms.

The greater number of researchers now, however, put little stress on precipitins in the production of these phenomena.

Nicolle has shown that a definite antibody is formed in the blood of animals injected with albuminous substances. By itself the antibody is quite non-toxic, but, in presence of its antigen, it develops a marked toxic power. The symptoms following a second injection of serum would thus be due to the union of the antibody formed in the organism with the antigen injected. The incubation period would be explained by the time required for the production of this antibody.

Friedberger believes that the anaphylactic phenomena are due to a definite poisonous substance, which he calls "Anaphylatoxin." This substance he has been able to prepare "*in vitro*" in various ways:

(*a*) By the action of normal guinea-pig serum on coagulated albumin;
(*b*) By the action of normal guinea-pig serum on normal horse serum; and
(*c*) By the action of normal guinea-pig serum on bacteria.

In these and other ways he can produce a toxin which, on injection into guinea-pigs, produces the phenomena of anaphylaxis. He has also done experiments to show the formation of anaphylatoxin "in vivo"—in the body of a guinea-pig.

Friedberger believes that there is no difference between bacterial and serum anaphylaxis if the amount of bacterial albumin is approximately near the amount of serum albumin used in the experiments. He points out that a very large quantity of bacteria are required to yield a small amount of albumin—*e.g.* one agar slope may only yield 1 mg.

—and for this reason anaphylactic symptoms are not usually seen with ordinary laboratory inoculations of bacteria. Friedberger supposes that " the characteristic poisoning in the infectious diseases results from the anaphylatoxin of bacterial albumin formed in small doses at the site of infection, and circulating in the organism." He has designated bacterial anaphylaxis as a " more extreme and acute form of infection," and infection as a " milder, more protracted form of anaphylaxis."

Turro and *Gonzalez* tried to determine which constituent of the serum causes anaphylaxis, and laid chief stress on the globulins.

Besredka, like Nicolle and many other workers on the subject, believes that anaphylaxis is due to a special antibody. In the injected serum there is, he supposes, a substance, " sensibilisinogen," which acts as an antigen. It produces the hypersensitive condition by inciting the organism to the production of an antibody, the " sensibilisin." This antibody has a specific affinity for the nerve cells and becomes fixed to them. The anaphylactic shock, according to Besredka, is due to the sudden wrenching of the sensibilisin from its union with the nerve cells which follows on the introduction of a fresh quantity of antigen into the circulation.

Richet employs a different terminology. The special substance produced in the body of the sensitised animal he calls " Toxogenin." This substance is not toxic in itself, but when a fresh quantity of antigen is introduced into the animal's body, the toxogenin unites with it to form a new substance, "Apotoxin," which produces the anaphylactic symptoms. (Toxogenin + Antigen = Apotoxin.)

Andrewes draws attention to the fact that a pronounced leucopoenia occurs after an intravenous injection of bacteria into an immunised animal, and he believes that this leucopoenia is closely connected with the symptoms of anaphylactic shock.

It is evident that the full explanation of Anaphylaxis is not yet in sight. The theories offered are endless and make an intensely interesting study; they are, however, beyond the scope of the present volume.

APPENDIX.

A. EHRLICH'S SIDE-CHAIN THEORY.

The basis of this theory was first set forth by Ehrlich in an article on cell nutrition in 1885. The contention was that nutritive substances, in order to be made use of by the body-cells, must first enter into chemical combination with certain elements of the cell protoplasm. The highly complex protoplasmic molecules of the cell he supposed to be made up of a central atom group ("Leistungs-kern"), upon which depended the specialised activity of the cell, and a multiplicity of other atom-groups, which he called receptors or side-chains, by means of which the cell formed chemical combinations with food substances.

Later, the theory was again brought forward by Ehrlich for the purpose of offering an explanation of the action of toxins on antitoxins, and of the mode of production of the latter in the animal body.

The side-chains or receptors of the body-cells are extraordinarily varied, and it is to this variety that Ehrlich's theory attributes the great diversity of the immunisation reactions against different infective agents.

Before any molecule—be it a food molecule, a molecule of infective material or a molecule of toxin —can enter into combination with the molecule of a body-cell, it is necessary that this cell should possess " suitable " receptors. (" Suitable " is the usually accepted translation of the German word " passende," but a more correct rendering would be " congenial," as the suitability must be mutual; the foreign molecule must be suitable for the receptor and the receptor for the foreign molecule. The idea is rather of an " affinity " or " twin-soul ! ")

Consider the case of infective material injected into the animal body. This infective material may give rise to the production of antibodies and is therefore called an antigen (see glossary). If the infective material can find no suitable receptors in any of the cells of the body, it cannot be assimilated; it is left free (" unfixed ") and the animal is said to be insusceptible or immune. This is a " want-begotten rest " which, Tennyson notwithstanding, is obviously an enviable possession.

If some of the cells do possess suitable receptors, the molecule of infective material becomes fixed to these and the sequel then depends (*a*) on the nature of the cells, and (*b*) on the nature of the foreign material (antigen).

(*a*) Consider first the importance of the nature of the cell. Let us suppose that tetanus toxin is the antigen, and that it finds suitable receptors in several kinds of cells. If, for example, it gets partly fixed to connective tissue cells and partly to nerve cells, the fixation will be detrimental to the nerve cells, but will apparently not affect the connective tissue cells.

This, according to the hypothesis, is due to the fact that tetanus toxin is a poison only for nerve elements, and that it is not poisonous for elements of the nature of connective tissue.

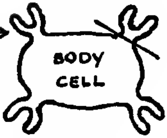
The fixation, therefore, only puts the toxin in a position to do injury ; whether or not injury follows depends on the constitution of the cell itself. A cell may thus be immune to an antigen from the fact that it possesses no suitable receptors for that antigen, or again, it may still be immune even after fixing the antigen, if its intimate constitution does not render it susceptible to the particular form of injury which this antigen is capable of inflicting.

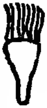
(*b*) The result following on the fixation of the antigen by the cell receptors depends largely on the nature of the antigen. If, for example, the antigen is a toxin, it will unite with certain receptors which have an affinity for a particular atom group of the toxin. Ehrlich's theory represents a molecule of toxin as being composed of two atom groups, a haptophore or affinity-bearing group and a toxophore or poisoning group. Now when the toxin has used up all the "congenial" or "suitable" receptors which it finds in the cells which have fixed it, Ehrlich supposes that the cells begin to suffer from the occupation of these receptors by a foreign substance, which thus shuts them out from their normal function of absorbing nourishment. The cells consequently set about producing similar receptors, not only in sufficient quantity to make up for those already occupied, but in great excess. These superabundant receptors get cast off from the cells and circulate in the body fluids where they now form the antibody known as antitoxin. This antitoxin, then, has simply an affinity for toxin, but has no other known function. When it comes in contact with toxin in the body fluids, it unites with this and neutralises its effect, thereby preventing any injury to the organism.

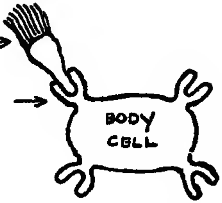
The point which here strikes one as curious is that the substance which, when attached to the cells, is the source of danger, is, when free in the fluids, the guarantee of safety to the animal. When detached from the cell, the superabundant receptors (antitoxin) are, so to speak, relieved of their original "métier," and may unite with the toxin without any risk of ill to follow. For explanation this has been compared to the action of a badly insulated lightning conductor. A more accurate comparison would be that of a key (toxin) and a lock which is not attached to any door (antitoxin). The key may be in the hands of the burglar and he may fit it exactly into the lock, but as long as the lock is a thing by itself, and is not connected with any door, the burglar is reduced to a state of harmlessness.

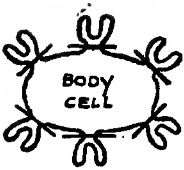
Ehrlich classes these antitoxins as *antibodies of the first order*—having only a haptophore or combining atom group.

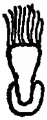
The following diagram shows Ehrlich's conception of the origin of these antibodies of the first order from the body cells, and also shows the hypothetical mode of union of toxin and antitoxin.







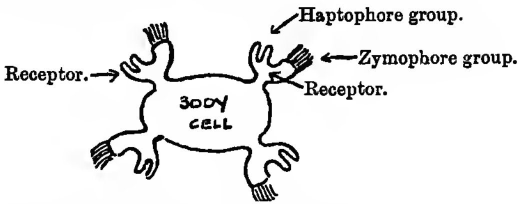




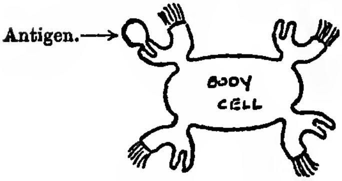
The above is what may be assumed to take place when the injected antigen is a toxin.

Other antigens, however, cause the formation of *antibodies of the second order*; *e.g.* if B. typhosus is injected agglutinins will be formed, and if a foreign albumin is injected, precipitins are formed. These agglutinins and precipitins resemble antitoxins in having an affinity-bearing (haptophore) group which unites with the antigen, but they differ from them in having also a functional (zymophore) group which exerts a demonstrable effect on the antigen after this union. The effect of the functional atom group may be to agglutinate the antigen (*e.g.* bac. typh.), in which case the antibody is called agglutinin, or it may be to precipitate the antigen (*e.g.* foreign albumin), in which case it is called precipitin.

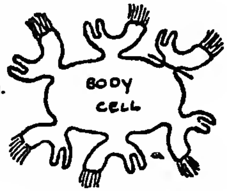
The diagrammatic representation of this is as follows :



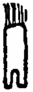
Immunising substance—bacteria or foreign albumin. →



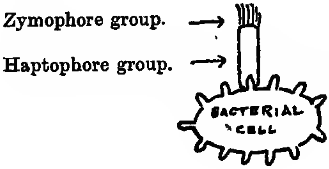
Union of bacterial or albuminous molecule with suitable receptor.



Cell forming receptors in excess.



← Receptor cast off from cell



Union of bacteria or foreign albumin with agglutinin or precipitin.

Still other antigens may incite to the formation of *antibodies of the third order*. These have no functional group which can operate directly on the antigen, but they have two haptophore groups, one (the antigenophile group) which unites with the antigen, and the other (the complementophile group) which unites with complement, and so allows the latter to operate on the antigen.

These antibodies of the third order are called amboceptors ; the bacteriolysins or haemolysins may be taken as examples. Thus, if red blood corpuscles from one animal are injected into an animal of a different species haemolysins will be produced, and if certain bacteria (*e.g.* vibrio cholerae) are injected, bacteriolysins will be produced.

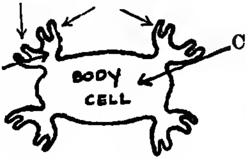
This is diagrammatically represented on p. 177.

The above explanation of Ehrlich's theory having been given, it is necessary to emphasise the fact that it is only *theory*. It may be useful in helping one to piece facts together and get a little order out of chaos, but it becomes a very dangerous hindrance the moment one forgets that it is all the merest speculation. Huxley has said that "Science commits suicide the moment it adopts a creed," and undoubtedly Ehrlich's theory has become a sort of " credo " to many Immunitists, especially in Germany.

There is much truth in Bordet's contention that this theory, by offering explanations before sufficient data had been accumulated, has really hindered the free development of investigation.

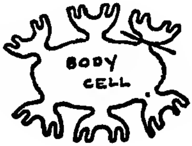
Bordet does not agree with certain points in

Haptophore groups.



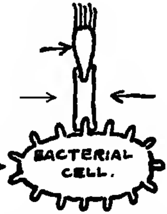
Receptor.

Cell with receptors; each receptor has two affinity-bearing groups.



Cell forming receptors in excess.

Receptor cast off.



Complement.

Amboceptor.

Receptor with two haptophore groups.

Antigen.

Union of amboceptor on the one hand with antigen, and, on the other hand, with complement.

Ehrlich's theory ; for example, to say that the antibody is identical with the receptor affected by the antigen, is to affirm something that has never been demonstrated as fact.

He also strongly objects to the manner in which Ehrlich's theory attributes all the diversity of immunisation phenomena to diversity of the molecule groups of the antibodies and takes no note of the varied nature of the antigens. He points out, for example, that in agglutination one has no right to say that it is the agglutinin which agglutinates and the bacteria which are agglutinated ; rather it is the complex bacteria plus agglutinin which is agglutinated by the salts in the fluid. The union of the bacteria with the agglutinin brings about a change in their molecular adhesion which renders them sensitive to the agglutinating effect of salts in the serum.

Similarly, Bordet objects to the view that the immune body in bacteriolysis is an amboceptor—*i.e.* has two combining groups. This is quite unproved. He considers that one ought to regard the complement as being fixed, not by the "complementophile" group of the "amboceptor," but by the complex formed by the union of the lytic body with the antigen. Just as the complex, agglutinin plus bacteria, has a modified molecular adhesion which brings about the liability to agglutination, so the complex, lytic antibody plus antigen, has a similar modified adhesion which brings about the capacity of complement adsorption. "This being so, there is no fundamental difference between sensitisers (Ehrlich's 'amboceptors'), and such other antibodies as antitoxins. There is no such thing as an amboceptor ; all antibodies are 'uniceptors.'"

Andrewes, in his Croonian lectures, has also sounded a note of warning. He says : "It is plain that a school of thought which translates the observed properties of a fluid into terms of personified entities, for which there is no actual chemical evidence, is not without its scientific dangers. To explain biological facts by a transcendental pseudochemistry may be useful enough as a working hypothesis, so long as we are content to regard it as such."

B. CHEMOTHERAPY.

In recent days this branch of the science of Immunity has grown with amazing rapidity and, especially since the introduction of salvarsan, has acquired a position of considerable importance.

The object of chemotherapy is to imitate Nature's method of overcoming an infection by the aid of substances which destroy the micro-organisms, whilst leaving the cells of the body of the host uninjured. To this end the chemical agent employed must be endowed with a much stronger affinity for the micro-organisms than for the body cells—must be more "parasitotropic" than "organotropic."

As early as 1902, *Laveran* and *Mesnil* had shown that arsenious acid is able to kill the trypanosomes in the blood of infected mice.

Then *R. Koch* had employed atoxyl in the treatment of sleeping-sickness with some success.

Uhlenhuth also had good results from the use of atoxyl in the syphilis of animals. *Salmon, Hallopeau* and others treated a number of cases of human syphilis with atoxyl, and claimed that it was efficacious. Optic atrophy, however, is a not infrequent sequela of the employment of atoxyl in the human subject, and this alone is sufficient to condemn it.

The honour of laying the foundations of chemotherapy, however, must be given to *Ehrlich.* He and his fellow-workers, by untiring research, have already been able to demonstrate the existence of three distinct classes of chemotherapeutic agents:

(1) The group of basic triphenylmethane dyes—parafuchsin, methyl violet, etc.;
(2) The group of benzidine dyes—trypan red, trypan blue, etc.;
(3) The group of arsenic derivatives—atoxyl, salvarsan, neosalvarsan, etc.

The experiments have been carried on chiefly against protozoal infections.

It soon appeared that the problem of curing an infected animal, with any of these chemical agents, was greatly complicated by the development of "resistant" races of the micro-organisms (see page 70). If the first dose of the drug was sufficiently large to kill off all the micro-organisms, the phenomenon, of course, was not observed. But if the dose was just small enough to allow a few to escape, these immediately fortified ("immunised") themselves against the drug and became invulnerable to its action. These resistant micro-organisms were then able to multiply, even in presence of the drug, and the astonishing discovery was made that this property of " drug-resistance " was transmitted from one generation to another. If, for example, trypanosomes in a mouse had become resistant to fuchsin (" fuchsin-fast "), a quantity of these trypanosomes could be inoculated into a fresh mouse and from it to another, and so on, for years, and finally it would be found that the trypanosomes still retained their immunity to the action of fuchsin.

The hereditary transmission of this acquired characteristic has been shown also in trypanosomes which have become " antibody-resistant." These protozoa may immunise themselves against the antibodies in the guinea-pig within the space of a few days, or against these antibodies " in vitro " within a few hours, and this acquired immunity will continue as a characteristic of this race of trypanosomes from generation to generation for an indefinite period.

According to Ehrlich's postulate, toxic agents cannot act on a micro-organism unless they are fixed by suitable receptors (*non agunt nisi fixata*).

Levaditi and Fraser have shown that the antibody-resistant trypanosomes do not fix the antibody, and it is probable that the analogy holds for the drug-resistant micro-organisms.

It is therefore easy to understand the hopelessness of endeavouring to cure an infected animal by repeated injections of a chemical substance if some of the invading micro-organisms have succeeded in becoming " resistant " at the time of the first injection. For this reason the object of Ehrlich's treatment is to achieve a *therapia sterilisans magna*—*i.e.* to kill off all the infecting micro-organisms by one large dose or two doses with short interval between.

The "resistance" acquired by micro-organisms is specific—a trypanosome which has become resistant to atoxyl is just as susceptible as other trypanosomes to the action of fuchsin or other trypanocidal substance.

Salvarsan. The most brilliant clinical application of these researches has been the introduction of salvarsan for the treatment of syphilis.

Ehrlich and Bertheim made the valuable discovery that the constitution of atoxyl was not, as had been supposed, that of a metarsenate, but was in reality that of a paramidophenylarsenate. With this knowledge it was then possible to prepare substitution products of atoxyl which would be less toxic to the host and more injurious to the parasite. Salvarsan or "606" is such a substitution product; it is "dioxydiamidoarsenobenzol." This being insoluble in water, the hydrochloride salt is that used for therapeutic injections. Before injection it is neutralised with sodium hydrate till the neutral salt forms and is precipitated; by further addition of sodium hydrate this precipitate is dissolved and the solution is injected into the patient. This represents the sodium salt of "dioxydiamidoarsenobenzol."

The great value of salvarsan is due to the fact that it is comparatively non-toxic; the doses required for therapeutic injection are not harmful to the patient, except in the rare cases where there is an idiosyncrasy against the drug. It has been pointed out, however, that the presence of some other infection, in addition to syphilis, seems to render salvarsan much more toxic for the patient, and warning is given against injecting this drug whilst a patient is suffering from another infective malady.

Apart from its application in syphilis, salvarsan has shown itself of great value in the treatment of many protozoal diseases, such as relapsing fever, framboesia, malaria, and sleeping-sickness.

Neosalvarsan. On account of the disadvantages attaching to salvarsan from its insolubility and the necessity of using the hydrochloride, Ehrlich has made laborious researches to discover a neutral salt which would be more soluble. Neosalvarsan has recently been introduced as a result of these labours. Its number is "914"—a fact which speaks for the work done since the discovery of "606."

It forms a yellow powder which is quite neutral and dissolves readily in water; the solution is then ready for injection, and the use of sodium hydrate, which formed an inconvenience in the case of salvarsan, is entirely dispensed with. Its toxicity is less than that of salvarsan, and it is thus permissible to use a somewhat larger dose and to shorten the interval between the injections.

It is still too early to report definitely on this substance, but Fildes and M'Intosh, who have been testing it in this country, are inclined to regard it favourably. They believe that owing to the lessened toxicity permitting a more intensive treatment, it is more frequently possible to attain a *therapia sterilisans magna* with this than with salvarsan.

Chemotherapy applied to Malignant Tumours. Quite recently Wassermann has published the results of some researches into the chemotherapy of cancer; these are at present far from complete, but they seem to offer a definite hope that the cure of this disease by chemical agents may one day be attained.

He found that the sodium salts of selenium and tellurium, when brought into contact with cancer cells, manifested a special affinity for these cells, and that eventually the metal became deposited within the cell body and had a disintegrative action. He then endeavoured to discover some substance which, when introduced into the circulation, would show an elective affinity for the tumour cells; by combining this with the selenium and tellurium salts he might cause it to act as a "carrier" or transporter of these to the tumour tissues, and in this way he hoped to destroy the latter. In experiments on mice he found that eosin could act as such a "carrier." He injected a combination of eosin and selenium into the circulation of cancerous mice and found that after a few days the tumour tissue softened and broke down, then the softened material formed a cyst, and finally the cyst was absorbed and the tumour annihilated.

Whether these experiments will lead to any similar discovery applicable in human therapeutics is still an open question.

GLOSSARY.

AGGLUTININS. (Lat. 'ad' =to, and 'gluten' =glue.) Antibodies in serum which possess the power of causing bacteria or blood corpuscles, suspended in a fluid, to adhere together and form clumps.

AGGLUTINOIDS. Agglutinins which have been so altered by heat or other agency that they have lost their power to agglutinate bacteria, but still retain their power of fixing themselves to the bacteria. In terms of Ehrlich's theory, they are said to have lost their agglutinophore, but kept their haptophore atom groups.

AGGRESSINS. (Lat. 'aggredior' =to approach in a hostile manner, to attack.) 'Attacking substances' produced by bacteria in the animal body; according to Bail, these substances have the power of paralysing the defensive mechanism of the body, in particular the leucocytes.

ALEXINE. (Gr. 'alexo' =to ward off, to turn away.) A term originally used by Buchner to denote the substance in serum which can cause haemolysis. Later, when Bordet had shown that two substances take part in this haemolytic process, he adopted the term to designate the substance in normal serum which must act along with the specific antibody in order that lysis may occur. The same substance is called 'cytase' by Metchnikoff and 'complement' by Ehrlich.

ALLERGIE. (Gr. 'allos' =otherwise, in another way, and 'ergon' =work. Allergie, therefore, literally means altered power of work.) The term applied by V. Pirquet to the altered power of reaction acquired by an animal as the result of inoculation. The alteration may be either in the direction of lessened susceptibility (immunity) or increased susceptibility (anaphylaxis).

AMBOCEPTORS. (Lat. 'ambo' = both, two together, and 'capio' = to seize. Amboceptor therefore literally means something which lays hold of two things.) The name given by Ehrlich to his group of 'antibodies of the third order,' which are specific serum substances, characterised by possessing, on the one hand, an affinity for the corresponding antigen and, on the other hand, an affinity for complement. (See Appendix, p. 176.)

ANAPHYLATOXIN. (Literally the toxin which brings about anaphylaxis.) The name given by Friedberger to the definite poisonous substance which he believes to be the cause of the symptoms of anaphylaxis. (See p. 167, Chap. on Anaphylaxis.)

ANAPHYLAXIS. (Gr. 'ana' = without, and 'phulaxis' = protection or security. Therefore anaphylaxis literally means a state of unguardedness.) A condition of hypersensitiveness produced in an animal by the injection of a foreign proteid.

ANTIBODIES. (Gr. 'anti' = in opposition. Therefore antibodies are literally substances which act in opposition to others.) Substances found in the serum of an animal in response to an infection or the injection of an antigen; *e.g.* antitoxins, agglutinins, precipitins, bacteriolysins, haemolysins, etc.

ANTIGEN. (Gr. 'anti' = in opposition, and 'gignomai' = to generate. An antigen therefore is literally something which generates an opposing body; it, however, does not generate but rather causes to be generated. The word is not happily chosen.) A substance which, when introduced into the organism, gives rise to the formation of an antibody. The antigen may be a toxin, a bacterial emulsion, a bacterial extract, an emulsion of blood corpuscles, a solution of albumin, etc.

ANTIGENOPHILE. (From 'antigen' and Gr. 'phileo' = to love, to have an attraction for.) Having an affinity for antigen. In Ehrlich's theory the amboceptor is said to have an antigenophile and a complementophile atom group.

Antitoxin. (Gr. 'anti' =in opposition, and 'toxicon' = an arrow—ultimately a poison for arrows.) The antibody found in the serum of an animal in response to the injection of a toxin or to an infection with toxin-producing bacteria. The antitoxin has the power of neutralising the corresponding toxin.

Bacteriolysins. (Gr. 'lusis" =a setting-free, from 'luo' =to loosen. A bacteriolysin is therefore something which loosens or breaks up bacteria.) Antibodies which, acting in conjunction with complement, have the power of lysing bacteria—*i.e.* of causing them to shrivel up into granules and ultimately to disappear. These substances are specific; the bacteriolysin for B. typhosus, for example, does not cause lysis of Vib. cholerae, and *vice versa*.

Bacteriolytic. Having the power of lysing bacteria.

Bactericidal. Having the power of killing bacteria.

Bacteriotropins. (Gr. 'trepo' =to turn or guide towards.) Name given by Neufeld and Rimpau to certain substances in immune serum which have the power of promoting phagocytosis. Unlike the 'opsonins' of normal serum they are thermostable—retaining their power after heating beyond 56° C.

Complement. (Lat. 'complementum' =that which completes. Ger. 'Komplement.') Name given by Ehrlich to the substance, present alike in normal and in immune serum, which acts in conjunction with an amboceptor. Synonymous with 'alexine' (Bordet) or 'cytase' (Metchnikoff). Destroyed by heating the serum at 56° C. for ½ hr.; the serum is then said to be 'inactivated.'

Complementophile. Having an affinity for complement.

Cytase. (Gr. 'kutos' =a hollow vessel or cell. The termination 'ase' is here used to denote a ferment as in diastase.) Term applied by Metchnikoff to the substance which acts in conjunction with the sensitiser (amboceptor) in producing haemolysis, bacteriolysis, etc. It is therefore synonymous with 'alexine' or 'complement.' Metchnikoff regards the cytase as being of the nature of a soluble ferment, formed within the phagocytes and only liberated on the destruction of these cells.

Endotoxins. (Gr. 'endon' = within.) Toxins contained within the bodies of bacteria.

Exotoxins. (Gr. 'ex' = out of, without, away from.) Toxins secreted by bacteria into the medium in which they are growing.

'Fixateur.' The name given by Metchnikoff to the immune substance which acts in conjunction with 'cytase' in bacteriolysis, haemolysis, etc. Synonymous with the 'sensitising substance' (Bordet) or 'complement' (Ehrlich).

Group-agglutinins. Agglutinins which act not only on the homologous bacteria, but also on other nearly related micro-organisms; *e.g.* agglutinins for B. typhosus may also act, though to a minor degree, on B. paratyphosus.

Haemagglutinins. Antibodies which cause the agglutination of red blood corpuscles. They are present in the serum of an animal immunised against the corresponding corpuscles.

Haemolysins. (Gr. 'haima' = blood, and 'luo' = to dissolve, separate, break up.) Antibodies in a serum which, when acting together with complement, have the power of 'lysing' red blood corpuscles. The haemolysins may be produced in response to the injection of blood corpuscles from an animal of a different species (specific haemolysins), but they may also be present to a certain extent in normal serum (normal haemolysins).

Haptophore Group. (Gr. 'hapto' =to fasten or bind, and 'phoreo' =to bear, carry. The haptophore group is literally one that carries the binding property.) In Ehrlich's side-chain theory the uniting or 'affinity-bearing' atom group.

Immune Body. Synonymous with amboceptor.

Inactivation. Term commonly applied to the process of heating a serum in the water-bath at 56° C. for ½ hr., in order to destroy the complement.

Labile. (Lat. 'labo' =to totter or waver.) Unstable; easily altered.

Lysis. (Gr. 'lusis' =a setting free; from 'luo' =to loosen, set free, break up, dissolve. Ger. 'auflösen.') The process by which bacteria or red blood corpuscles have their protoplasm broken up or 'loosened' so that they undergo an alteration in shape and, in the case of the blood corpuscles, the loss of their haemoglobin, which appears to escape through a rupture in the stroma.

Macrophages. (Gr. 'makros' =large, and 'phagein' = to eat. Literally, the large eating cells.) One of the two classes into which Metchnikoff divides the phagocytes. In this class are included the large amoeboid cells of the spleen pulp and of the lymphatic glands and omentum, certain endothelial cells and some cells of the neuroglia and connective tissue.

Meiostagmin Reaction. (Gr. 'meion' =smaller, and 'stagma' =a drop.) The test recommended by Ascoli for the diagnosis of malignant tumours, etc., by the method of counting the number of drops formed by a given quantity of fluid after an antigen and antibody have united in this fluid.

Microphages. (Gr. 'mikros' =small, and 'phagein' = to eat. Literally, the small eating cells.) One of the two classes into which Metchnikoff divides the phagocytes. This class contains the polymorphonuclear leucocytes.

Negative Phase. The name given by Wright to the period which may follow the injection of a vaccine, during which the opsonin-content of the blood is temporarily diminished.

Opsonins. (Gr. ‘opson’ =sauce, seasoning, pl. ‘opsonia’ =rich fare, dainties.) The name given by Wright to certain substances in blood serum which prepare bacteria for ingestion by phagocytes and so increase the amount of phagocytosis.

Opsonic Index. The ratio of the number of bacteria which can be ingested by a given number of phagocytes, in presence of a patient's serum, to the number ingested by the same number of phagocytes in presence of a normal serum.

Phagocytes. (Gr. ‘phagein’ =to devour, to eat, and ‘kutos’ =a cell. Literally, the eating cells.) The name given by Metchnikoff to the amoeboid cells which are capable of ingesting bacteria, blood corpuscles, and other formed elements.

Phagocytosis. The process of ingestion of bacteria, blood corpuscles or other formed elements by phagocytes.

Phagocytic Index. The number of bacteria ingested by leucocytes under the influence of serum, divided by the number of leucocytes; *e.g.* if the number of bacteria in a series of 20 leucocytes is found to be 100, the phagocytic index will be $\frac{100}{20} = 5$.

Pfeiffer Reaction. The transformation into granules, etc., of certain bacteria, such as Vib. cholerae or B. typhosus, when injected into the peritoneum of an animal immunised against the particular micro-organism.

Polyvalent Serum. (Gr. ‘poly’ =many, and ‘valere’ = to be worth. Literally, worth many, equal to many.) A serum prepared by immunising an animal with a number of strains of a micro-organism (*e.g.* streptococcus) with the object of producing substances which will be protective against all strains.

Polyvalent Vaccine. A vaccine prepared from several strains of a micro-organism, such as streptococcus or gonococcus.

Precipitins. (Lat. 'praecipitare' = to cast down headlong.) Antibodies found in the serum of animals immunised against bacteria or against solutions of animal or vegetable albumins, which have the power of producing a precipitate in a clear solution of the particular albumin, or in a clear culture filtrate of the bacteria, against which the animal has been immunised.

Receptors. (Lat. 'recipere' = receive. Literally, receivers.) According to Ehrlich's side-chain theory, the protoplasm of the living cell is composed of a central atom-group which determines the cell functions, and of various molecule-groups (called side-chains or receptors) which serve chiefly for the nutrition of the cell. These receptors have very varied affinities for different nutritive substances, and some show an affinity for injurious substances, such as toxins, bacteria, etc. The contention is that it is from these receptors, produced in increasing quantity, that the various antibodies are formed.

Sensitising Substance. ('Substance sensibilisatrice.') The name given by Bordet to the substance in immune serum which, acting along with alexine, gives rise to haemolysis, bacteriolysis, etc. Synonymous with 'amboceptor' (Ehrlich) and 'fixateur' (Metchnikoff).

Serum Disease. ('Serum Krankheit.') The name given by Schick and V. Pirquet to certain symptoms—skin eruptions, swelling of glands and joints, oedemata, etc.—which may follow the injection of a serum from an animal into another of different species.

Side Chains. (See Receptors.)

Stimulins. (Lat. 'stimulo' = to goad, annoy; to stimulate to any action.) The name given by Metchnikoff to substances in serum which incite or stimulate phagocytes to ingest bacteria, etc.

Toxins. (Gr. ‘toxon’ =an arrow. The drug with which the arrow was poisoned was called the ‘pharmakon toxicon.’) The true bacterial toxins are secretion products given off by the bacteria into the medium in which they are growing.

Toxoids. Toxins which have been so modified by long keeping or by heat, that they have lost their toxic properties, and retain only their power of uniting with the corresponding antitoxin.

Vaccine. (Lat. ‘vacca’ =a cow, ‘vaccinus’ =pertaining to a cow.) The name ‘vaccination’ was given by Jenner to the process of inoculating the ‘cow-disease’ (vaccinia) into human beings, and the term ‘vaccine’ was retained by Pasteur to denote any attenuated virus inoculated in order to produce protection against the fully virulent virus.

Zymophore. (Gr. ‘zume’ =a ferment, and ‘phoreo’ = to carry.) In Ehrlich’s theory the zymophore group of the agglutinins and precipitins is the functional atom group which brings about agglutination or precipitation. Besides the zymophore group, the agglutinins and precipitins possess a uniting or ‘haptophore’ group.

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